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Pulmonary Deposition of Dust as Affected by Electric Charges on the Body

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Introduction

FOR many years workers in numerous fields have endeavored to define the mechanism or mechanisms responsible for the deposition of air-borne particulate matter in the respiratory tract. It is often difficult to see why apparently similar biological systems in atmospheres of quite similar aerosols react in very different ways. Factors such as aerosol solubility, particle size, chemical activity, surface structure, hydroscopicity and many others have been extensively investigated.[1-6] It seems to be the consensus that penetration and retention are governed by a number of interrelated factors. One factor, recently considered, is the electrostatic charge on the particles.[7] Essentially unstudied has been the effect of electrostatic charge on the body. Either or both of these might have a strong and varying influence on particle penetration and deposition.

Areas directly related to this subject are relatively unexplored, but investigations of the effects of "air ions" on the biological system and physical-chemical experiments with the behavior of charged particles have essentials in common. Several reviews[8-13] of the work on the effects of "air ions" (actually a misnomer, as it includes particles much larger than ions) infer that the presence of a very small electrostatic charge may influence a number of physiological phenomena—including the respiratory function. Certain effects attributed to air ions may actually have been due to much larger charged particles. No one has explained the basic mechanisms involved in air-ion effects.

Physicists and chemists have carried out well-designed experiments and calculations to determine the actions of charged particles under various conditions.[14-18] This makes it possible to predict, when certain parameters are known, the behavior of a given aerosol in any given set of electro-physical conditions. This line of investigation unfortunately has not been carried into the realm of the biological system. Certain characteristics of biological systems make it difficult to draw paralleling predictions with similarly charged surfaces and particles.[19] Landwehr[7] has suggested that certain dusts seem, on generation, to have a predilection for either positive or negative mass charges. He further postulates that the dusts producing the pneumoconioses seem to be negative when put into aerosol form, while innocuous dusts tend to be positive.

The human body is capable of conducting electricity; it is common for an individual to notice the presence of several thousands of volts of static electricity. It is known that almost any means of forming an aerosol will result in the charging of a great number of particles.[17, 18] In 1947, Wilson[20] suggested that a unipolar aerosol might increase the lung retention factor; however, this postulation apparently has never been subsequently investigated. These factors suggest a need for the investigation of the interaction of the electrostatic fields on both the particle and the animal body.

This paper concerns itself with the amount of aerosol found in the lungs of laboratory animals exposed while in a charged and uncharged state. An animal at a high potential of either positive or negative polarity demonstrates a significant difference from an animal at ground potential relative to the deposition of dust in the lungs.

Experimental Procedure

General Plan

It was thought best to attempt to obtain an answer to the basic problem by exposing three sets of animals simultaneously in the same exposure chamber. Of the three groups, one was charged to a high negative potential, one to a high positive potential, and one remained at ground (or zero) potential. Dust was generated in the chamber, and after an exposure period of two hours, the lungs were subjected to chemical analysis for the test aerosol.

Exposure Chamber

The chamber was a modified biological hood (manufactured by Kewaunee Manufacturing Company, Adrian, Michigan). which was sealed,

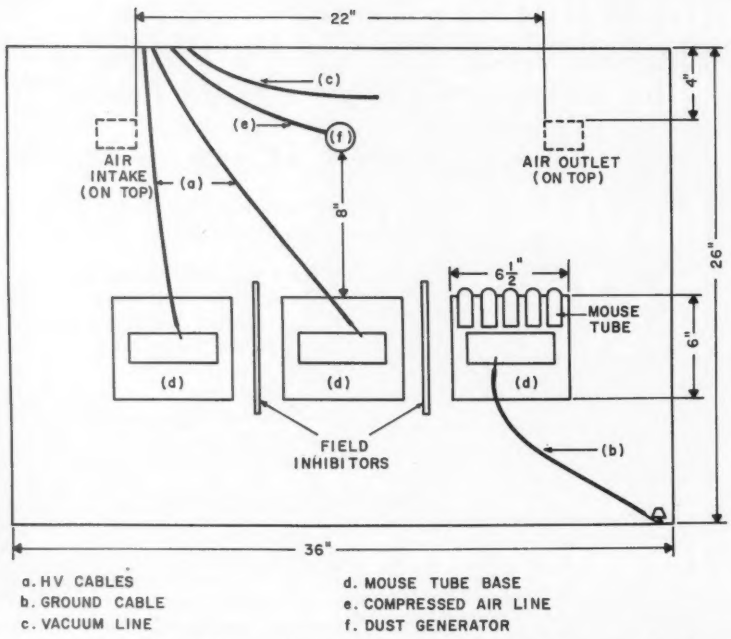

FIGURE 1. Floor plan of dust exposure chamber. Ten mouse tubes were placed on each base. The groups are numbered 1, 2 and 3 from left to right.

and the air circulation system modified as shown in Figure 1. Circulation was maintained at low velocity by means of a blower and duct system situated on top of the chamber. The only outside air supplied was the small amount introduced by the dust generator. A slight negative pressure was maintained by a vacuum line near the floor of the chamber. The chamber was lighted, and a glass front and glove ports made adjustments possible during exposures.

Because the type of air circulation and the general configuration of the chamber would possibly permit uneven concentrations of aerosol at the three exposure positions, test runs with membrane-filter aerosol samplers (Millipore Filter Corporation, Bedford, Mass.) at each of these points were set up. Each sampler tested a given volume of chamber air, and the collected dust was then quantitatively analyzed. As an additional precaution, each position was used an equal number of times at positive, negative or ground conditions. The chamber itself was grounded.

Test Aerosol

Potassium chromate was used as the test aerosol in all exposures. This material was dissolved in distilled water (5 grams per 100 ml water) and placed in a dust generator, which operated on somewhat the same principle as those used by Wilson and LaMer.[20] A potassium chromate dust was produced with the following distribution of particle size (observed with light and electron microscopes).[21]

Size Range (microns)	*Percentage*
0.1–0.5	80
0.5–1.0	10
1.0–3.0	5
3.0–5.0	5

Generation of dust was accomplished by producing a fine mist with the use of submerged high-velocity jets and the subsequent slow travel of this mist up a 17½- by 1⅝-inch pyrex tube.[21] Atomized solution was partially filtered by the layer of liquid and bubbles above the jets, then subjected to evaporation as it moved up the tube. As the larger droplets fell back into the solution reservoir, smaller droplets evaporated, leaving very small particles of the solute. Only relatively dry particles were found near the top of the generator.[15]

Visual observation by means of intense light

indicated that the generated dust dispersed from the mouth of the tube radially, some swirling out and down, with the greater part of the cloud moving toward the intake opening of the air circulation system. The generator was operated throughout the exposure period because a portion of the chamber air was constantly being removed, there was precipitation on the chamber walls, and it was felt that an "aging" factor entered into the electrostatic properties of the aerosol.[15] As the level of the liquid in the generating device fell, fresh solution was added. Several times during the course of the fifteen runs, new potassium chromate solution was made; thus, the concentration of the solution changed slightly from run to run. This and other factors made it apparent that the dust concentration in the chamber probably varied somewhat over the period of fifteen runs.

Experimental Animals

White Swiss mice, obtained from a colony closed for over twenty years, were used throughout this work. These mice were placed in pyrex tubes (see Figure 2), immobilized, yet allowed to breathe and attain a high electrostatic charge, with the tail and charged (or grounded) plate forming the contact points. The critical factors in this arrangement were:

1. The animal's nose had to protrude beyond the tube opening (due to the absence of electrostatic charge inside a hollow semiconductor).
2. The tail must remain outside the tube and in good contact with the charged or uncharged plate.
3. Breathing could not be overly restricted by packing the animal too tightly.
4. The size of the mice was limited due to the dimensions of the containing tubes.

An obvious consideration in this type of exposure study is that every effort must be made to have each of the three groups of mice in any given run as nearly alike as possible. This was managed by using mice six to nine weeks old and weighing between 25 and 30 grams. Within a run, all mice were of the same colony, age, and sex and weighed within 3 grams of one another.

A total of 450 mice were used, 30 mice per run with 10 mice in each charge group. Tubes were used as restraining devices in order to maintain the desired electrical condition on each mouse. Any other containers, such as wire cages, large open-topped vessels, and the like, would certainly have had a precipitative effect on the nearby dust, thus changing the dust concentration some distance from the animals. It was desirable to maintain a high potential on the animal in as near "normal" conditions as possible.

Lung Tissue Analysis

Potassium chromate was chosen as the test aerosol for two reasons: (a) its high degree of water solubility makes it suitable for use in the previously described aerosol generator, and (b) there are very sensitive methods available for its quantitative analysis in tissue samples. A modified combination of the methods used by Urone[22] and Baetjer[23] for the microdetermination of chromium in biological tissues was used with only two basic departures:

1. Ashing of the lung tissue was accomplished by the combined effects of nitric and sulfuric acid and the operation of a hot plate at a final temperature of 400° to 500°F. Erlenmeyer flasks afforded a long period of refluxing.
2. Centrifugation of the final oxidized sample, just prior to the diphenylcarbazide color development described by Urone, was found unnecessary for the purposes of this experiment.

The sensitivity of this test was found to be

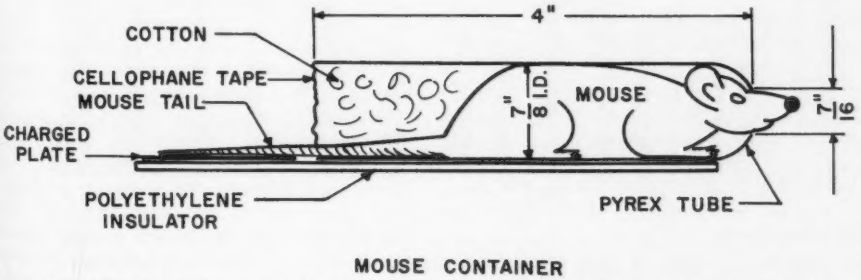

FIGURE 2. View of mouse in container. The mouse tails and tubes were secured to the base with tape.

0.005 microgram of chromium, as also seen by Urone. In most cases the chromium content of the lungs varied approximately 1 to 3 micrograms, and the reagent, plus unexposed mouse lung chromium content, was less than 0.05 microgram; hence, contamination, when it occurred, was always of such great deviation that it was obvious.

The mice were simultaneously sacrificed with chloroform immediately after the exposure period. Within 15 minutes of cessation of exposure the lungs and approximately 2 mm of trachea were removed with avoidance of contamination by outer body surfaces and the esophagus. Excess blood was washed off the lungs although the chromium content of the blood after exposure for two hours was found to be nil. A detailed description of the analytical procedure may be found in previous publications.[21, 22]

High Voltage Source

High voltage was obtained from an RF power supply of the type normally used to supply the final anode potential to cathode ray tubes. This was modified to give a ground-free supply by isolating the secondary winding of the air-cored transformer. The output voltage was divided by two equal resistors which had their center point grounded. The current through these resistors was high compared to any leakage current encountered in practice so that under all circumstances equal positive and negative potentials were available.

A meter was provided at the high-voltage terminals, and the unit was operated from a commercially available, variable voltage, regulated power unit (Model 710B, Hewlett and Packard Co., Palo Alto, California). Voltages (referred to ground) were available in the range 0 to 8000 volts. In the first three exposures, 2000, 5000, and 7000 volts were used in that order. No appreciable differences in pulmonary deposition of the aerosol were seen with these voltages, and 4000 volts was then arbitrarily decided upon for use in all other runs.

Aerosol Charge Analysis

Throughout this investigation it was apparent that the test dust was to some degree charged. Efforts were made to secure some information as to the polarity of the dust cloud in the chamber. A rough approximation of this factor was made in two ways:

1. A large, aluminum foil plate was connected to an electrostatic voltmeter (Weston Electrical Instrument Corp., Newark, N. J.), and both were charged to approximately 2500 volts, positive or negative potential. As the dust in the chamber fell on this plate, it caused a neutralization of the imposed charge. The neutralization process was evaluated by observing the voltage drop on the meter. The voltage fall was measured, timed and compared with the voltage fall seen with the opposite polarity and with that seen when no dust was in the chamber.
2. Two aluminum foil plates of the same size and charged several thousand volts positively and negatively were exposed to the chamber dust for a given length of time. Dust concentrations on the two plates were then quantitatively determined.

These methods were repeated many times and were used as checks against one another. After several trial attempts, both methods were developed to the point where reliable approximations of the relative percentages of the quantities of positive and negative particles were obtained. Since these methods depend upon the particles' being charged, it was not possible to determine the quantity of neutral particles.

Humidity and Temperature Evaluations

Two types of psychrometers were used in checking the temperature and humidity of the chamber during a run. One was operated by the hand squeeze-bulb method (Bendix Aviation Corp., Baltimore, Maryland), and the other was a motor-driven, fan-type device (E. H. Sargent & Co., Chicago, Illinois); there was good correlation between the readings of the two instruments. The nature of the dust generating device and the variations in room conditions suggest the possibility for a variable factor for each run. Both humidity and temperature were checked for the full exposure period on several runs.

Results and Discussion

Examination of Figure 3 and Table I establishes the fact that in each of the fifteen runs the group of mice which were exposed while at ground potential showed higher pulmonary chromium accumulation than either the positively or negatively charged groups of mice. Further examination indicates that the positive and negative groups, with respect to one another, seem to run high and low in a random fashion. This graph also indicates the chamber positions of each group during each run. Positions in the chamber are 1, 2, and 3, left to right (see Figure 1), and it should be noted that the relative positions of the "charge groups" were rotated through each of the three positions five times during the course of the 15 runs.

TABLE I

Average Concentration, Range and Standard Deviation of Chromium Content in the Lungs for Each Run

Run number	Micrograms of chromium											
	Positive group				Negative group				Ground group			
	High	Low	Average	S.D.	High	Low	Average	S.D.	High	Low	Average	S.D.
1	1.77	.75	1.45	.34	1.93	1.23	1.70	.21	2.35	1.16	1.94	.36
2	2.63	1.31	1.76	.37	2.17	1.31	1.85	.28	2.54	1.47	1.99	.40
3	2.26	1.31	1.78	.33	2.26	1.03	1.55	.37	2.72	1.61	2.00	.32
4	1.47	.75	1.08	.22	1.38	.63	1.02	.27	1.53	1.03	1.22	.18
5	2.54	1.31	2.09	.65	2.72	1.47	1.90	.39	3.20	1.93	2.51	.35
6	2.26	1.31	1.74	.28	2.26	1.23	1.73	.33	3.10	1.31	2.26	.51
7	2.63	1.31	1.93	.63	2.54	1.61	1.96	.28	2.81	1.77	2.11	.29
8	1.31	.75	1.00	.19	1.53	.63	1.01	.30	1.47	.82	1.06	.20
9	1.23	.48	.79	.20	1.08	.55	.87	.20	1.31	.82	.93	.16
10	1.93	1.08	1.55	.24	2.63	1.23	1.72	.39	2.72	1.23	1.95	.43
11	1.77	1.08	1.37	.20	2.09	1.08	1.51	.28	2.54	1.61	1.93	.26
12	1.09	.57	.82	.16	1.08	.66	.83	.13	1.30	.62	.92	.22
13	.68	.36	.50	.11	.75	.43	.54	.11	1.69	.43	.88	.41
14	.88	.68	.80	.07	1.08	.55	.74	.19	1.08	.68	.86	.11
15	2.17	1.08	1.65	.31	1.53	1.03	1.32	.15	2.54	1.47	2.04	.32
Averages	1.77	.94	1.35	.29	1.80	.98	1.35	.26	2.19	1.20	1.64	.30

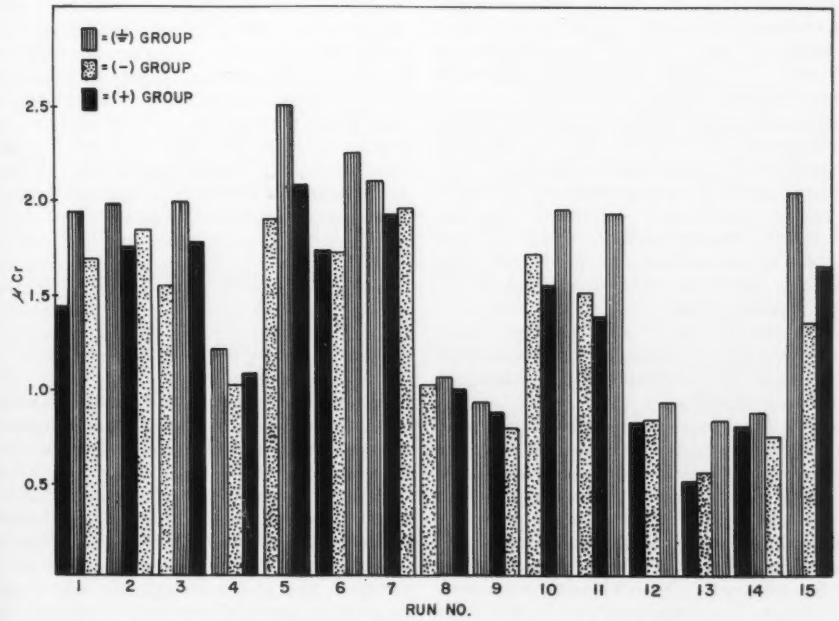

FIGURE 3. Concentrations of chromium in mouse lungs. Each bar represents 10 mice of the group of 30 per run. Within each group the order of placement of the bars denotes chamber position 1, 2 or 3 from left to right.

Statistical analysis of the experimental results serves to amplify the apparent findings shown on the graph. It was found that the positive and negative groups showed average concentration percentages of 17.2 and 17.0 respectively less than the ground group. These ranged from 5.7% to 43.2% less than ground for the positive groups and from 4.7% to 38.6% less for the negative group. By means of the small sample test for difference (with paired items)[24] it was found that there is a highly significant difference between the concentrations of both positive and negative groups from the ground group. This method also showed that there was no significant difference between the positive and negative groups themselves. A two-factor analysis of variance test[25] comparing the effects of types of charge with the effects of chamber placement served to indicate that there is no single or interaction effect due to the chamber placement or that the rotation of groups in placement positions nullified any effect.

Standard deviation determinations were calculated on each group of ten mice (see Table I) as well as on the total experimental group of 450 mice (in this case a standard deviation of the standard deviations). All concentration values were found to be well within 2.58 standard deviations, and only 27% of the concentrations in all mice were outside one standard deviation for their respective groups.

A summary of the findings is then as follows:

1. Grounded mice consistently accumulated significantly more dust in the lungs than either positively or negatively charged mice.
2. Positive and negative groups of mice accumulate varying amounts of dust with respect to one another—either may be high in any given run. The data offers no explanation for this.
3. Chamber placement (which was evenly alternated among charge groups) accounted for none of the differences in pulmonary dust accumulations.

Since the validity of a statistical analysis in this type of investigation is suspect if adequate controls in several areas are overlooked, it is appropriate at this point that certain means of control be reviewed. Several obvious factors which would most assuredly influence aerosol inhalation are taken into account and made relatively insignificant by two elements of experimental design. These elements are: (a) the three groups in any one run were exposed to the same dust concentration, and (b) the final results were obtained by calculating the percentages of the two low groups as related to the single high group. In this manner, humidity, dust concentration, temperature, average mouse size, metabolic rate, and other elements will obviously vary from run to run, but they should be nearly the same for any single run of three groups. Several factors generally believed to have an influence on dust exposure studies will be discussed because these are important to a general understanding of experimental conditions rather than because they have had a direct bearing on the results of this investigation. One important variation, the proportions of charged and uncharged particles of both polarities, did occur and will also be discussed.

Humidity and temperature checks showed surprisingly little variation between runs or during any given run. It would seem that the nature of the dust generator would preclude this observation, since it depends on evaporation for the production of a dry particulate aerosol. However, several checks on both factors during the course of runs of two hours showed only an average 4% rise in humidity and a 3°F rise in temperature. The dust generator and/or the presence of high potentials seem to exert a stabilizing influence. The very dry (10% RH), cool, compressed air supply was the only "fresh air" admitted into the chamber. A quantity of water was inadvertently spilled in the chamber during one humidity check, and the humidity immediately increased 20%; within ten minutes it fell to its previous level. The average humidity and temperature for all checks was 45% and 74°F respectively, and the ranges were 37% to 50% and 72° to 79°F.

Three aerosol concentration checks showed the following average concentrations:

Position	*Micrograms Cr/ft³*
1	256
2	254
3	251

These results, together with the evidence from the statistical analysis, would indicate that the aerosol concentration within the chamber was fairly homogeneous.

A much more interesting parameter, and certainly more pertinent to this and subsequent experiments, was that developed by an investigation of the charge on aerosol particles. Of the two methods used to demonstrate the relative quantities of positive and negative charges, the most accurate was that utilizing the electrostatic voltmeter—although good correlation was seen with the other. The average results for four checks by the first method are as follows:

Average voltage drop, plate charged positively	
With dust in chamber	6.35 volts/sec
Without dust in chamber	3.43 volts/sec
Difference in voltage drop rates	2.92 volts/sec
Average voltage drop, plate charged negatively	
With dust in chamber	8.07 volts/sec
Without dust in chamber	3.25 volts/sec
Difference in voltage drop rates	4.82 volts/sec

It can be seen that there was a 46% increase in drop rate with the positively charged plate and a 60% increase in drop rate for the negatively charged plate. This amounts to a difference of 23% in rates between the positive and negative conditions. It seems reasonable then to assume that the predominant charge on the dust falling on the charged plate was positive, and that there was approximately 23% more positive than negative dust (assuming a dust of homogeneous particle size and charge per particle). In spite of certain obvious limitations in this line of reasoning, follow-up checks on dust polarity, using the second method, show good correlation. As previously described, two plates are oppositely charged, exposed to the dust, and quantitatively analysed for chromium. The average results were as follows: (a) positively charged plate, 0.040 mg Cr; (b) negatively charged plate, 0.050 mg Cr. Therefore, approximately 20% more dust was deposited on the negative plate indicating a predominantly positively charged dust. It was unfortunate that neither of the two described methods afforded a means of obtaining the percentage of "neutral" dust particles.

Several other checks on polarity by the second method brought out a rather interesting observation. The predominating polarity of the dust cloud alternates from positive to negative. Several authors[14, 17, 18] have indicated the possible means by which similarly generated dusts show this polarity variability. It would seem likely that, in this investigation, the most probable mechanisms involved were the varying concentrations of potassium chromate in the solution and the effect of "aging" (probably a matter of slow reduction of hexavalent chromium). It seems plausible that a dust of changing polarity would have a noticeable effect on the intake of that dust by highly charged animals.

It can be seen from Figure 3 that the concentrations of dust in the positive and negative groups are frequently almost the same, but more often either the positive or negative group may show a greater concentration. This shift does not statistically vary with position; hence, some more subtle factor must be involved. It seems quite possible that the shift may be due to the changing polarity of the dust cloud. The lack of a practical measuring device prevented the drawing of real conclusions on this matter, but work is now being planned which may cast some light on this problem.

In considering further work along these lines it becomes obvious that the only unassailable finding thus far is that an electrostatic body charge on mice has an effect upon the inhalation of a chromate dust. This immediately suggests additional investigation into more basic mechaisms interacting between the animal and the dust. Since almost nothing is known about these reactions, it is only possible to postulate in broad terms what the causes might be for the observed effects.

It is quite difficult to set up an electro-physical model for the purpose of an explanation of the action of electrostatic charges on an animal body. However, it may be said that, in general, the body can be viewed as a charged conductor. Taking the inner lung surface into consideration, the body may be thought of as a charged hollow conductor and will then show the electrostatic properties demonstrated in Faraday's classic "ice pail experiment." Briefly, the fundamental laws derived from this experiment state that there can be no charge on the inner surface and no force field in the inner space of a hollow, charged conductor. Furthermore, the walls of this conductor contain no lines of force, and the field is most intense near pointed projections of the body (as the animal's nose). These principles should operate similarly on the charged body of the animal. Therefore, as soon as dust particles (charged or uncharged) enter the respiratory tract, the electrostatic charge on the body itself will have no effect on the particles. It should be noted here that, should the particles be charged, on impingement on the inner respiratory tract surface, electrons may then "migrate" to or from the body surface causing a slight increase or decrease (depending on polarity) of the body surface potential.

It is therefore quite probable that the only influence of body charge on the dust particles is due to that force which is felt by a particle as it approaches the highly charged animal nose. This force may act in the following ways:

1. If the particle is of the same polarity as the animal, there will be a repelling force tending to drive the particle away from body surfaces and inhalation.
2. If the particle is of opposite polarity, an attracting force will tend to move the particle toward outer body surfaces and possible impingement.

3. If the particle is at zero potential (neutral), it will be unaffected by the body charge except for the relatively slight attraction caused by the creation of an "image charge" as the particle moves very close to the charged body.

In this investigation, the variations in concentrations of the two charged groups may have been caused by some difference in the quantity of charge on positive or negative particles, or by the relative difference in the influence of possibilities 1 or 2.

Another factor which may play an important part in this phenomenon may be that of the elaboration of charged moisture droplets in the form of exhaled breath from a highly charged animal. It has been observed[21] that a human who has been charged to approximately 9000 volts will exhale moist air capable of charging or discharging an electrostatic voltmeter by some 3000 to 4000 volts. These moisture particles could come in contact with, and thereby charge or discharge, aerosol particles. An investigation is now in progress which will attempt to establish a definite relationship between charged and uncharged animals and aerosols.

Summary

It has been shown that the placing of a high electrical charge on groups of laboratory animals significantly influences their ability to inhale and subsequently show a pulmonary accumulation of particles of a test aerosol. More specifically, this has been shown true with potassium chromate dust and groups of animals maintained at positive and negative potentials of 4000 volts. In each of fifteen aerosol exposures, another group of animals was maintained at ground or zero potential, and in each exposure this group was found to have a significantly higher concentration of dust in the lungs than either the positive or negative groups. It is therefore suggested that the electrostatic condition of an animal may have a strong influence on its reaction to an environmental aerosol in either natural or experimental situations. It is also possible that the electrostatic charge on an aerosol particle may have a great deal to do with its entrance into and subsequent deposition in the respiratory tract.

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Retention and Effect of Radioactive Particulates in the Lung

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THE possibility of exposure to radiation as the result of the inhalation of radioactive particulates has led to renewed interest in the dynamics of deposition and retention in the lung. (Related terms are defined as follows: *deposition* concerns the transfer of suspended particles from the air to the inner surfaces of the lung during the process of respiration; *retention* refers to the time interval during which this material remains in the lung after it is deposited; *lung burden* is the amount of material present in the lung at any given instant of time; and *clearance* is the rate at which the lung burden decreases as a result of *transport* to other regions of the body or of *elimination* from the body by the excretory mechanisms.) Thompson, Parker, and Kornberg,[1] for example, have pointed out that three criteria are necessary for the estimation of a maximum permissible concentration of a radio-isotope in the environment; (a) the critical organ and its critical burden must be known; (b) the possible sources from which the organ burden is derived must be known; and (c) the quantitative relation between environmental concentration and corresponding organ burden must be estimated. In practice, one cannot predict which organ will be critical; rather, one calculates the critical environmental concentration for each of the more probable organs in turn. The critical organ is, by definition, the one which corresponds to the lowest critical environmental concentration, and this lowest figure then becomes the maximum permissible concentration. The lung plays an especially important role in such a calculation, since it may on the one hand turn out to be itself the critical organ, or it may on the other hand alter the effective exposure of some more peripheral organ by acting as a reservoir from which small but important concentrations of isotope are maintained in the blood over long periods.

In both of these roles the lung behaves in a unique fashion which resists prediction. In the case of radium, for example, it is possible to compute the total radium content of the body from observations on blood, urine, and feces, provided that the blood radium levels are in equilibrium only with radium stores in organs other than the lungs, whereas the calculation breaks down if any appreciable reservoir of undissolved radium exists in the lung. Similarly, the calculation of the amount of ionizing radiation which can be absorbed by the lung without damage is complicated by the fact that the radiation dose delivered by inhaled radioactive particulates is: (a) not distributed uniformly through the lung tissue, but is very great in cells adjacent to the particles and small or absent in other cells more distantly located, and (b) the dose is directly proportional to the retention time of the particles, a value which varies over extremely wide limits with changes in either the isotope, its particular chemical compound, or its physical property (1½ hours to 6 months; LaBelle and Brieger, 1959).[2] In view of these complications, the direct determination of a safe level for radioactive particulates in the lung by empirical methods is very much in order.

Biological descriptions of toxic effects in an organ can be, and very often are, related to environmental parameters such as the atmospheric concentration and the time of exposure, without knowledge of the actual tissue dose involved. If in addition to biological effects one wishes to examine the kinetics of retention and transport, it is desirable to start the experiment with lungs the original burden of which is known as accurately as possible. Experience has shown that foreign particulates can be suspended in saline solution and injected into the lungs of rats with a reproducibility (standard deviation) of the order of plus or minus 10%, whereas replicate animals exposed to the same material by inhalation show a variability in initial dose which is three to five times as large (LaBelle, Booth, and Barrett, 1953).[3] Intratracheal injection was therefore adopted as the basic method for this study.

A sample of commercial uranium dioxide was finely ground by several passes through a hammer mill and then classified by liquid sedimentation

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into a series of particle-size fractions. A bulk sample of the fraction whose mean size was six microns was placed in the nuclear pile at Oak Ridge and exposed to neutron bombardment for 18 months. At the time of its removal from the pile the isotope composition of this material was essentially that of "spent fuel." The material was allowed to stand for several years, during which time most of the short-lived isotopes decayed out, leaving only the longer-lived isotopes such as uranium, plutonium, and strontium. At the time of use, the material exhibited a half-life of about 15 ± 5 years and a specific activity of about 2,000 disintegrations per minute per microgram. The emission was largely beta-gamma, with something less than 5% of alpha activity present.

This material was available as a suspension of particles in water. Working stock suspensions in physiological saline solution were prepared by dilution, and so-called injection preparations were prepared in small quantities as needed by dilution of the working stock suspension. In each case an attempt was made to obtain an activity of 100,000 dpm in three milliliters of suspension; each injection preparation was assayed before use so that the radiation later recovered from an individual animal could be related to the assay of the preparation actually used. The range of such assays over the entire experiment was roughly from 9,000 to 11,000. The injection of this material into the lung followed precisely the method described by LaBelle (1953, p. 1749)[4] except that instead of an adjusted dose of 30 milligrams of uranium dioxide per kilogram of body weight, each rat received a fixed dose of 10,000 dpm contained in 0.3 milliliters of physiological saline solution.

Groups of such animals were sacrificed at the following times after the injection: 1, 2, 4, 7, 17, and 24 hours (1 day); 2, 4, 7, 14, and 30 days (1 month); 2, 3, 6, 12, and 18 months. At least 20 animals were sacrificed initially at each point, followed by additional animals in those cases where the scatter was unusually great, so that the standard error of the mean of each group was brought into the range of plus or minus 5 to 10 per cent of the mean. At each point a corresponding group of exposed rats was sacrificed for histological examination. Each rat was killed by an overdose of Nembutal and the lung removed immediately (*i.e.*, within 2–3 minutes). Each lung was weighed and placed in a pre-counted porcelain crucible and covered with concentrated nitric acid. After standing overnight, the acid, containing most of the lung tissue in solution, was evaporated and the residue ignited for 30 minutes at 600 to 700°C, after which the crucible containing the ignited lung tissue was counted under a thin-window Geiger counter tube. After correcting the total count for background and blank reading on the crucible, the count was divided by the assay figure for the preparation injected (*i.e.*, by approximately 10,000 dpm) to obtain the percentage of the starting dose recovered in the tissue at the time of autopsy. Counting times were adjusted to keep the counting error below 5% of the total count, which required five minutes for the early samples and 18 hours for the later preparations. Since the results appeared to be at least roughly exponential in character, individual observations were combined into groups by taking a geometric rather than an arithmetric average, a procedure which appears to be entirely justified by the fact that the standard deviations remained essentially constant when calculated on this basis. These means are recorded in Table I.

TABLE I

Retention of Activated Uranium Dioxide Particles in Lungs of Rats
(Initial dose 10^4 dpm/rat = *ca.* 5 micrograms/rat)

Time	% of initial dose retained	Time	% of initial dose retained
0 hours	100.0	7 days	45.4
1 hour	88.7	14 days	46.4
2 hours	90.4	1 month	41.9
4 hours	96.4	2 months	39.0
17 hours	71.6	3 months	19.2
1 day	83.1	6 months	11.3
2 days	76.3	12 months	8.6
4 days	78.2	18 months	4.5

In addition, four survivors examined at 28 months show an average of 1.1% of the initial dose still retained in the lung.

Simple inspection of the data reveals that a single exponential expression is inadequate to represent the transport of the uranium dioxide particles out of the lung. It is a characteristic of the exponential curve that equal fractions are converted in equal times starting at any portion of the curve whatsoever. In the data of Table I, however, it is seen that the amount retained falls from 100% to 50%, half the material being eliminated, in a little less than seven days, whereas at the other end of the Table it requires six months (18 months minus 12 months) to reduce the residual by the same fraction (4.5%/8.6%).

A two-phase exponential system seems more appropriate to the data, but unfortunately there is no simple and direct method for fitting such a system. One can, however, start with the probably unwarranted assumption that the starting dose is sequestered, immediately upon injection, into two mutually exclusive and completely independent compartments, each of which clears ex-

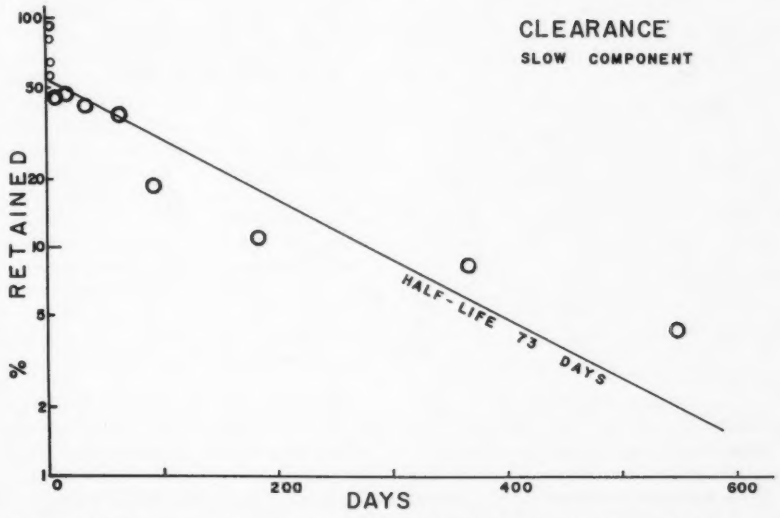

FIGURE 1. Slow component of clearance of radioactive particulates from the lungs of rats given uranium dioxide by intratracheal injection.

ponentially at its own specific rate. If one of these rates is significantly greater than the other, the late changes will be controlled almost entirely by the slower rate. Proceeding on this basis, one fits a first approximation to the slow regression by conventional methods, but using only the later figures, in this case those from seven days and beyond. This regression is extrapolated backwards to zero time and the predicted retentions at 1, 2, 4, etc., hours are calculated and subtracted from the observed retentions at these time intervals. The differences are taken to be due to the presence of the fast component in the system, and a first approximation to the fast regression is accordingly fitted. The fast component is then extrapolated forward to obtain the effect of this component over the entire series, and a new set of residuals is obtained by subtraction which now permits a second approximation to the slow component, this time over the entire series. This process of successive approximation is repeated until no further change results in the equations; in the present case, the fifth solution was identical with the fourth, and the process was halted at this point. The resulting equations are:

Fast component: $\text{Log Ret}_{\text{fast}} = 1.6075 - 0.4173\ t$

Slow component: $\text{Log Ret}_{\text{slow}} = 1.7375 - 0.00248\ t$

These expressions have the following meaning: 30.5% (antilog 1.6075) is eliminated from the lung with a half-life of 17 hours, and 54.6% (antilog 1.7375) is eliminated with a half-life of 73 days. This leaves a balance of about 15% of the dose not accountable in the regressions, which may suggest a third component but more probably is simply accumulated experimental error.

Because of its nature, the slow component can be shown directly in a plot of log retention against time, as in Figure 1, in which the exponential with a half-life of 73 days remains linear. The fast component, however, can only be shown as a linear function in logarithmic residuals, or else as a curved function, as in Figure 2, in which the 15% error term mentioned above has been combined with the slow regression term. While neither curve is an especially good fit for its data, this must not be allowed to obscure the fact that they are nonetheless useful approximations. For example, there can be little question that two separate mechanisms are operating in clearing the lung, quite aside from the question whether either or both of them are truly exponential in character or not. Again, the exponential fitted to 18 months of observation may yield a highly erroneous prediction of what to expect in the next 18-month period but it still represents an effective and efficient summary of the data over the range for which it was calculated.

Specifically, the exponential regressions derived

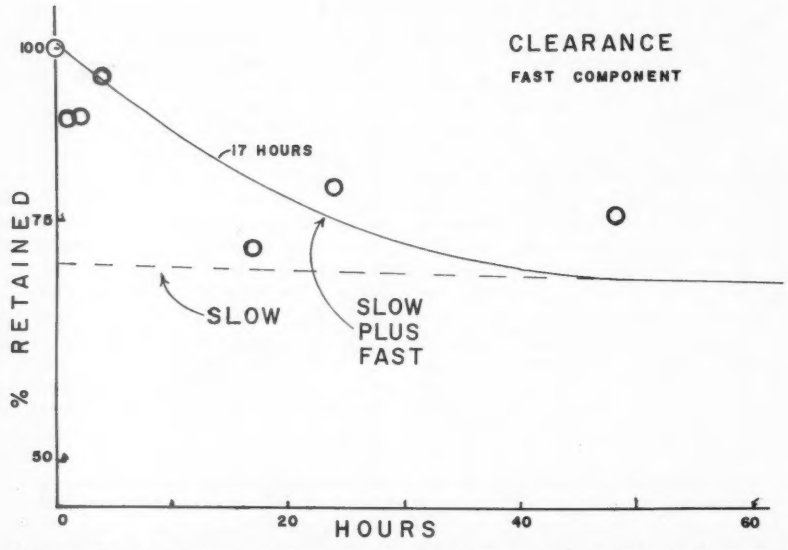

FIGURE 2. Fast component of clearance of radioactive particulates from the lungs of rats given uranium dioxide by intratracheal injection.

in this manner can be integrated between any two limits such as 0 to 1 year, to obtain the total accumulated exposure of the lungs in DPM-Days. When this is done for the two regressions separately and the results added, we obtain a value of 671,000 DPM-Days as the total exposure over the first year, and about 60,000 as the total over the second year. Taking one MEV per disintegration as a reasonable average for the isotopes known or believed to be present, and taking the weight of the rat lung as one gram, which is approximately correct for a fluid-free lung at the time of the average exposure, one obtains a value of 15.5 rep as the total absorbed dose during the first year of the experiment, and about $\frac{1}{10}$ this amount during the second year. This is, fortuitously, almost identical with the accepted tolerance value for lung tissue (Morgan, Snyder, and Ford, 1956).[5]

The various factors and mechanisms which are involved in the fast component of clearance from the lung have already been discussed in earlier papers. Brieger and LaBelle[6] have shown that the material leaving the lung during this phase passes through the trachea into the gastro-intestinal tract, whence it is excreted in the faeces. LaBelle and Brieger[2] showed that the fraction of the total which is involved in this rapid phase can vary between wide limits (from 10% of the total to 90% of the total) as a result of changes in particle size, chemical composition, and total lung burden. The importance of this fast component in exposure to radioactive particles cannot be overemphasized. For example, if we accept the original premise of sequestration into independent compartments, it follows that a particle in the slow compartment exposes the tissue for an average of 73 days, while the same particle in the fast compartment exposes the tissue for only 17 hours. Any portion of the total which can be shifted from the former to the latter therefore has its radio-toxicity reduced in the proportion of 73/0.70, or almost 100-fold, so that even a small increase in the amplitude of the fast component means an important reduction in radiological hazard.

The interpretation of the slow component is less obvious. At least a part of the material involved in this phase may be eliminated in a relatively harmless manner through the gastro-intestinal tract (Gross, 1953),[7] while the remainder almost certainly reaches the blood stream. In any case, the total exposure of the lung tissue is derived almost entirely from this slow component of the clearance curve.

Since this total exposure falls almost precisely at the accepted tolerance level, the biological findings in these animals become of special importance. Two independent lines of evidence suggest that animals exposed at this level do not develop any signs of damage related to their radiological exposure.

The first of these lines of evidence is obtained

from the lung weights recorded for those animals which were used in establishing the retention curves. It can be shown that the weights of lesion-free lungs in rats lie almost uniformly between the limits of 0.50 and 0.60% of the total body weight of the animal, and that this fraction changes very little throughout the lifetime of the rat. Almost any pathological process in the lung will increase this figure by a large amount; congestion in the blood vessels without any demonstrable escape of fluid into the airways can raise the weight by 50%, while consolidation, fibrosis, or frank edema can raise the weight of the lung to 5–7 times its normal value. All this makes the lung-weight to body-weight ratio a valuable and quantitative index of the progress of a pathological process in the lung.

The mean value of this ratio for each group of rats is recorded in Table II. It can be seen that except for an unimportant and transient rise immediately following the injection, the lung weights remain within the normal range throughout the lifetime of the animals.

The second line of evidence derives from the independent groups of animals examined by serial autopsy. These animals were prepared and sacrificed in exactly the same way as the others except that the extirpated lungs were prepared for histological examination instead of radioactivity measurements. In most cases, the lungs were fixed by intratracheal instillation of fixing fluid. This was done either before the opening of the thoracic cage, so as to avoid overdistention, or after removal of the intact lung from the thoracic cavity. The instillation of the fixing fluid, even in an amount that distended the lung to more than four times its initial volume, did not produce any artifactual alterations such as rupture or emphysema. In some instances, the lungs were fixed by immersion in the fixing fluid and trimmed as soon as the periphery was hardened. Bouin's and Zenker's fluids were used, but better fixation was obtained with Zenker's fluid and especially with the Held-Romeis mixture. Only sagittal parahilar sections were chosen for examination. This procedure has the advantage of showing on one slide the entire lung from apex to base. Specimens were stained with hemalum-eosin, and with Masson's trichrome stain when an increase in connective tissue was suspected.

Microscopic comparison of the experimental and the control specimens failed to reveal significant differences; when unidentified slides were examined, it was difficult to differentiate between exposed and control tissue. In some cases the pathologic changes seen in the treated animals were actually less severe than those seen in the controls.

TABLE II

Changes in Lung Weight in Rats Exposed to Radioactive Particulates (15 rep)

Time	Lung-weight to body-weight ratio %	Time	Lung-weight to body-weight ratio %
0	0.55		
1 hour	0.83	7 days	0.73
2 hours	0.80	14 days	0.72
4 hours	0.73	1 month	0.75
7 hours	0.67	2 months	0.59
17 hours	0.75	3 months	0.53
1 day	0.80	6 months	0.55
2 days	0.71	12 months	0.56
4 days	0.72	18 months	0.66

Bronchial Tree: The epithelium showed no significant pathologic changes, and the lumen was always patent except where intercurrent respiratory disease had occurred. Here an exudate of neutrophilic cells was observed. The stroma showed no deviation from normal except that there was seen a slight increase of eosinophilic cells, lymphocytes and lipofuscin-laden cells in the experimental specimens.

Alveolar Walls: Irregular thickening was present in both the experimental and the control lungs. This was not due to fibrosis or its precursor stages, the stroma was infiltrated with histiocytes and monocytes. The lesions were discrete and sporadic, and found focally or generalized; they involved either the entire contour of an alveolus or only an arc of its periphery. This cellular infiltration was present mostly in the subpleural region, with no evidence of topographic predilection. No exudate cells other than histiocytes and monocytes were seen. Occasionally there was also a sprinkling of neutrophilic, eosinophilic, and plasma cells; lymphocytes were absent. In general, the increase in cellularity was accompanied by a widening of the respiratory capillaries, with or without hyperemia. In some cases, the findings could be traced to residual intra-alveolar edema or hemorrhages.

Luminal Blockage: In both the experimental and control specimens, histiocytes and monocytes had entered the lumina, either singly or in groups. When found intraluminally, the monocytes were of a large type. When found in groups, they tended to coalesce either by their tapered ends or by their flanks. No inclusions and no debris of any kind were visible in the cytoplasmic body of these cells. Derivative structures, such as foreign-body giant cells or miniature granulomata, were not in evidence. Luminal blockage was seen in scattered and separated alveoli or appeared sporadically in groups of alveoli or in patches.

The lesion was more frequent in areas with residual hemorrhages and/or edema.

Only two findings that were absent in the control specimens were encountered in experimental specimens. These were (a) foam cells and (b) enlargement of the juxtabronchial lymphocytic patches.

Foam Cells: These cells were located only in the alveolar lumina, singly, or in groups leading to a pseudo-epithelial arrangement. They were occasionally scattered diffusely through the tissue, but in most cases they were seen focally, most of them subpleurally and especially juxtascissurally. The cytoplasm was not always "foamy" but looked occasionally rather glassy, with slight indication of vacuolization. The cells contained submicronic, highly refractile, angular grains which did not stain. These grains may represent residues of incompletely washed-out fixing fluid.

Peribronchial Lymphocytic Agminations: These cell aggregations, demonstrable only in the largest bronchi, were slightly wider in the experimental than in the control specimens.

Neoplasia: None of the findings in the experimental or in the control specimens showed or suggested tumor formation. Of special significance is the fact that the lungs of our rats which survived for three years following exposure revealed neither neoplasia nor morphologic changes that might be interpreted as precursors of a neoplastic process. The lungs, as a whole, were essentially normal except for a defense reactivity consisting of perivascular mantling with lymphocytes and histiocytes and cell types derived from the latter. Some of the respiratory cells in the alveoli had undergone metamorphosis into alveolar phagocytes. Occasional monocytes were seen intra-alveolarly; scattered eosinophilic cells were present. This microscopic picture reflects an incompletely healed and somewhat attenuated chronic inflammatory reaction of long duration that had failed to lead to fibrosis.

Conclusions and Summary

A 5-microgram dose of uranium dioxide particles placed in the lungs of rats is eliminated in two stages. During the first stage, about one-third of the material is removed within the first day or two (half-life 17 hours) while the remainder is eliminated much more slowly, with a half-life of 73 days, so that small but measurable amounts can still be recovered during the second year. With a specific activity of 10,000 dpm in five micrograms of material, the integrated radiation exposure to the lung during the first year is almost exactly equal to the accepted tolerance dose of 15 rep per year. No signs of radiological damage could be detected in any of the animals exposed to this dose, either during the year in which the exposure occurred or during the following year, when the effective exposure was for all practical purposes negligible. This confirms the accepted view that a dose of 15 rep within one year is without effect, since the observation period, though extending only to a total of two years, is very nearly equal to the life span of the test animals. It is worth noting, however, that the amount of radioactive material required to yield this tolerance level is rather greater than is implied by the maximum permissible intake tables of Morgan, Snyder, and Ford.[5] In other words, the Morgan tables are conservative. This is to be expected a priori, since our two-phase elimination curve would correspond very roughly to a single exponential with a half-life of about 36 days, whereas the Morgan tables are based on an assumed half-life in the lung of 120 days. Unfortunately, there is evidence to suggest that at least some insoluble particulates are cleared from the lungs much more slowly, with half-lives up to six months. In such cases the figures in the Morgan tables would be too high. This does not imply that the Morgan tables are not valuable, but only that they need to be used with due allowance for known departures from their premises.

Acknowledgments

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Evaluation of Radiation Hazards Created by Thoron and Thoron Daughters

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Introduction

THORIUM and its compounds have had limited industrial uses for many years. More recently, alloys of thorium with magnesium or aluminum have been developed and are being used in special fields. Such applications, and the potential demand for thorium as a fertile material for breeder reactors, make it desirable to study the potential health hazards that may be created by these and other industrial uses of thorium.

As in the uranium series, a dense, radioactive gas (Rn^{220}), generally called thoron, appears about midway in the thorium decay chain. Unlike uranium, purification of thorium does not remove all of the immediate precursors of thoron, as the 1.9 year Th^{228}, which decays directly into the 3.64 day parent of thoron, remains in the purified product. In addition, the longest lived nuclide in the decay chain (Ra^{228}) with a half-life of only 6.7 years builds in rapidly. Therefore, like thorium ores, commercial thorium will always be a source of thoron, with its emanating power depending on the time lapse since the material was purified. The work described in this report was undertaken to evaluate theoretically the relative significance of thoron and its daughters as sources of lung radiation doses and to develop methods for measuring atmospheric concentrations of thoron daughters.

Calculations of Atmospheric Concentrations

The decay of thoron and thoron daughters, which is shown in Figure 1, is quite different from radon and radon daughters.[1] From Figure 1, three assumptions are made which simplify the following calculations and which will not produce erroneous results. These assumptions are: (a) There is a 100% decay of ThA to ThB; (b) due to the very short half-life of ThC′ (3×10^{-7} sec.), it may be neglected in the calculations and ThC may be considered to decay 33.7% of the time by a 6.07 Mev alpha and 66.3% of the time by an 8.78 Mev alpha; and (c) since the energies and specific ionizations of alpha particles are very much greater than those of beta particles, the dose delivered to the tissue may be calculated on the basis of alpha energy only.

The dose delivered to the lungs and bronchi by both thoron and thoron daughters was calculated for a 40-hour workweek in a concentration of 10 micromicrocuries of thoron per liter in equilibrium with its daughters. It was assumed in these calculations that the total deposition of the thoron daughters in the lungs was 25 per cent of which one-half was deposited in the upper respiratory tract, and that deposited daughters were retained in the lungs until they decayed to ThD. All physical data used, such as weight and volume of lungs, breathing rates, and weight of bronchial tissue, were obtained from *The Report of the Committee on Permissible Dose for Internal Radiation*, 1958 revision.[2] In calculating the dose from thoron alone it was further assumed that the concentration of thoron in the lungs was the same as that in the outside air and that the daughters produced in the lungs were deposited there and retained until they decayed to Pb^{208}. While this assumption is probably erroneous to some extent (see Chamberlain and Dyson[3]), any error introduced into the calculated dose would be on the safe side. The total potential alpha energies (the total alpha energy delivered in the decay of all alpha-emitting atoms present to stable lead[4]) of thoron and of thoron daughters were then summed for the equilibrium concentration stated above and converted to rads to obtain the tissue doses listed in Table I. This table shows that the calculated tissue dose delivered by thoron daughters is much greater than that delivered by thoron only. For example, the calculated dose to the whole lung from thoron daughters is 700 times that from thoron. Therefore, the remainder of this paper will deal in great part with thoron daughters. The same situation was found to be true with radon and its daughters.[5]

Effects of Ventilation

Ventilation is the most effective means of reducing the concentrations of radon daughters in mine atmospheres.[6, 7] This should also prove true for thoron daughters as the only difference in the effect of ventilation should be that due to their different half-lives.

* On detail from Radiological Health Division, U. S. Public Health Service.

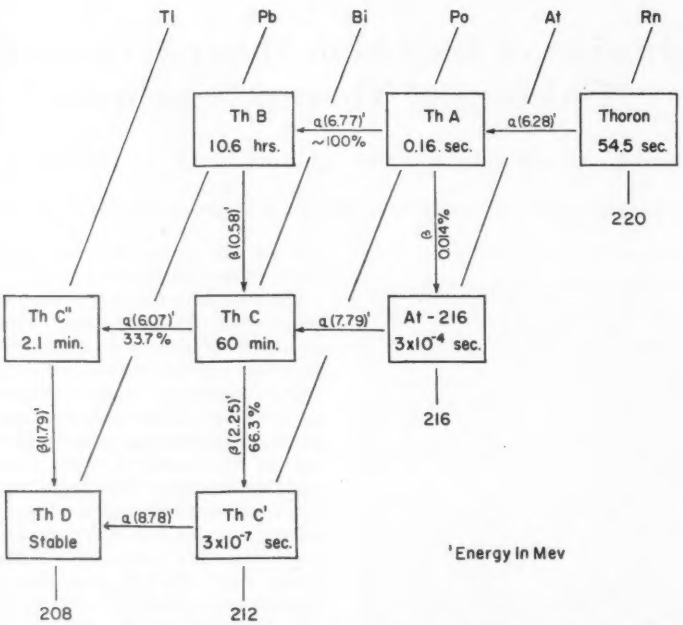

FIGURE 1. The radioactive decay of thoron and its daughters to stable lead-208 (ThD).

TABLE I

Radiation Dose to Respiratory Tract from Thoron and Thoron Daughters

Body organ affected	From inhaled thoron		From inhaled thoron daughters	
	Rads per week	Rems per week	Rads per weeks	Rems per week
Lungs	0.000049	0.00049	0.0349	0.349
Bronchi	0.000095	0.00095	0.874	8.74
Bronchi (1 cm in diameter)	0.00111	0.0111	—	—

10 $\mu\mu$c of thoron per liter at equilibrium with its daughters; RBE of 10 for alpha particles.

The effect of ventilation on thoron daughter concentrations may be calculated theoretically from the general equation[8]:

$$\frac{\lambda_i N_i}{\lambda_1 N_1} = \frac{\lambda_2 \lambda_3 \cdots \lambda_i}{C_2 C_3 \cdots C_i} \qquad (1)$$

In equation (1), N is the number of atoms in the volume described, λ_i is the disintegration constant of the i^{th} term and other terms respectively, and $C_i = \lambda_i + q/V$ where q is the cubic feet per minute of fresh air introduced into volume V in cubic feet. Hence, q/V will be equal to the number of air changes per minute. Figure 2 shows the theoretical percentage of equilibrium of each of the thoron daughters with thoron at the steady state under various ventilation conditions with removal only by decay and ventilation. With a very small fraction of an air change per minute

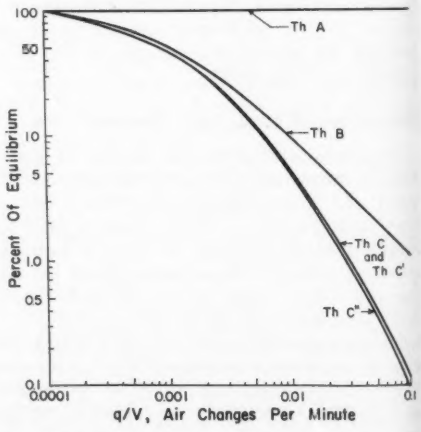

FIGURE 2. The effect of ventilation on the equilibrium concentrations of the various thoron daughters.

the concentrations of ThB and succeeding daughters are decreased considerably due to the long half-life of ThB. This shows that ventilation removes thoron daughters from a workroom atmosphere much more effectively than it does radon daughters.[8, 9] However, due to the short half-life of thoron, practical rates of ventilation will have very little effect in reducing the atmospheric concentration of thoron. For example, an air change as often as once every 54.5 seconds will only reduce the thoron concentration to half the level that would be obtained with no ventilation.

The Potential Alpha Energy and the Working Level

Sufficient biological data are not available to determine fully a maximum permissible concentration for radon daughter products. Until such data are available a working level of 1.3×10^5 Mev of potential alpha energy per liter has been suggested for radon daughter products (RaA, RaB, and RaC).[10, 11] Since the amount of energy delivered to the tissue is a primary factor in determining injury, it is reasonable to assume that one working level of thoron daughters should, as in the case of radon daughters, be equal to 1.3×10^5 Mev per liter of potential alpha energy.

The potential alpha energy of each thoron daughter may be calculated for any given concentration by multiplying the number of atoms of each isotope present in the air by the total alpha energy released by each atom during its decay to stable lead. Such calculations show that the potential alpha energy contributed by ThB will be much greater than that of the other daughter products. This is due to the long half-life of ThB which means that it will always be the most abundant isotope present in the air at any given time. For example, at equilibrium, the ratios of ThA to ThB to ThC are roughly 1:240,000:20,000. As the equilibrium ratios of the daughters change with a change in rate of ventilation (Figure 2), the fraction of the total potential alpha energy delivered by ThB will also vary. Therefore, the error in considering only the potential alpha energy delivered by ThB will vary, dependent upon the ventilation rate, as is shown in Table II. The error is quite small in all cases and this procedure eliminates the necessity of knowing the equilibrium ratios between the daughters.

Figure 1 shows that the number of atoms of ThB present can be related to the activity of ThC when an equilibrium is established. The disintegration rate of ThC can be measured by counting the alpha activity on the filter paper (as shown in Figure 1, ThB decays by beta emission and thus will not interfere). Then, from the measured activity of ThC, the number of atoms of ThB can be calculated by employing the equations for transient equilibrium.[12] The total potential alpha energy can then be obtained by multiplying the potential alpha energy per ThB disintegration (7.84 Mev) by the number of ThB atoms present.

As ThB has a half-life much longer than the half-life of ThC, only a few ThC disintegrations per minute are equivalent to a large amount of ThB potential alpha energy. This would indicate that the counting rates of samples collected in the field will be quite low. Therefore, it would seem that the ionization chamber survey instrument used in the field to measure radon daughter activities will be too insensitive to measure thoron daughters with acceptable precision.

It has been the practice to cite radon daughter concentrations in terms of multiples or fractions of the working level, where a working level is considered as 1.3×10^5 Mev of potential alpha energy per liter.[13] This is suggested also as the procedure when measuring thoron daughter concentrations. The term, working level, is thought to be more meaningful when reported to mining and industrial personnel than values given in micromicrocuries.

In order that these calculations may be less rigorous when used in the field, a number of terms have been combined into a graph which is shown in Figure 3. These terms include: the potential alpha energy of ThB as related to the number of ThC disintegrations per minute; a correction for decay of the ThB (C_d); and that one working level is equivalent to 1.3×10^5 Mev of potential alpha energy per liter. (At a working level of one there are 1.3×10^5 Mev/7.84 Mev = 1.65×10^4 atoms of ThB per liter. This number of ThB atoms is equivalent to 17.6 dpm or eight micromicrocuries per liter. *Therefore, at equilibrium eight micromicrocuries per liter of thoron is equal to one working level of thoron daughters.* This

TABLE II

Error in Calculated Potential Energy at Various Ventilation Rates

Ventilation (air changes per min.)	% of Equilibrium		Error caused by ThC initially present (activity counted after 5.6 hrs)
	ThB	ThC	
0.00	100	100	8.2 Percent
0.001	53.0	48.50	7.5 "
0.005	18.0	12.70	5.8 "
0.010	10.0	5.40	4.4 "
0.050	2.1	0.40	1.5 "
0.10	1.1	0.11	0.8 "

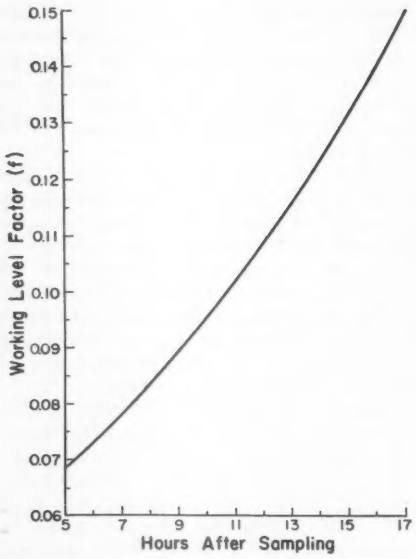

FIGURE 3. The working level factor curve for thoron daughters.

value is considerably less than that for radon which is 100 micromicrocuries per liter in equilibrium with its daughters.)

In practice, an atmospheric sample is taken on filter paper and the alpha activity measured at an appropriate time. This alpha activity is primarily due to the ThC which has grown in from the collected ThB. From the measured alpha activity the disintegrations of ThC per minute per liter are calculated, and this activity is multiplied by the factor obtained from Figure 3 to obtain the atmospheric concentration of ThB in terms of fractions or multiples of a working level.

Sampling, Collecting and Counting

In order that the previous calculations and the working-level factor (Figure 3) be valid, the ThC in a sample (a membrane filter is suggested as a collecting medium) must be in radioactive equilibrium with ThB when counted. The time after collection for this equilibrium to be reached will vary depending upon the initial state of equilibrium in the atmosphere. If the initial state is at equilibrium, the time will be zero which will increase to 5.6 hours for a sample void of ThC. Even though the latter case will probably never be encountered, it is usually quite difficult to determine the state of equilibrium in atmospheric samples and it therefore is good practice to let 5.6 hours elapse between the time of collection and the time of counting. This insures counting a sample in complete equilibrium and, as uranium is present in many thorium-bearing ores in North America, it will also permit any radon daughters to decay. However, if necessary, the contribution of the radon daughters can be calculated by obtaining a decay curve of the sample.

When the atmospheric contaminants are radioisotopes whose half-lives are short compared with the time of collection, it is necessary to take into account the build-up and decay of such elements on the filter paper during the sampling period. However, for thoron daughters this problem is not critical as the element of interest (ThB) has a comparatively long half-life of 10.6 hours. It was determined experimentally that no error was produced by a 30-minute sampling time, and, theoretically, a sampling time as great as 100 minutes will not introduce a serious discrepancy. Therefore, much longer and hence larger thoron daughter samples may be collected than is possible in the case of radon daughter samples.

The Contribution of Thoron to the Total Dose Delivered

From the preceding calculation, it was established that the dose derived from thoron is insignificant (less by a factor of about 700) in comparison to that dose delivered by the thoron daughters. Also, from the half-life values of thoron and thoron daughters and from Figure 2, it is obvious that ventilation has a great influence on reducing daughter concentrations in the air, but will have practically no effect on the reduction of thoron itself due to the very short half-life. Therefore, it is possible that if the thoron concentration is very high and there is considerable air movement, the thoron dose to the lungs may be a significant fraction of the total dose received. Figure 4 is a graph showing the contribution of thoron to the total dose delivered at various ventilation rates. The total dose, D_t, can be calculated from the thoron daughter dose, D_d, by equation (2) if the ventilation rate is known:

$$D_t = \frac{D_d}{1 - x} \qquad (2)$$

In equation (2), x is the fraction of the dose contributed by thoron and is obtained from Figure 4.

Even though the ventilation rate may be such that thoron contributes a sizable percentage to the total dose, the initial concentration must be quite large for this to have any significance. For example, in an atmosphere containing thoron daughters at a concentration of ten times the working level and an equilibrium amount of

thoron, the contribution of the thoron to the lung dose will be only 0.015 times the working level. For the thoron dose to become significant, it seems reasonable that it would require a thoron concentration which would deliver a dose to the lungs equivalent to at least one working level which can be calculated to be 5,360 micromicrocuries per liter. It is reasonable to assume that this level would be exceeded only under extreme conditions.

Suggested Survey Procedure

Atmospheric thoron daughter concentrations may be determined by following the procedure given below which is based on the preceding calculations and discussion:

1. Draw a known quantity of air through a filter paper. A total volume of 50 to 100 liters is suggested.
2. After sampling, allow the filter sample a minimum of 5.6 hours to reach equilibrium. Then determine the number of alpha disintegrations per minute by using a calibrated alpha detecting instrument. (Gas flow proportional counters or alpha-sensitive scintillometers are satisfactory.)
3. By dividing the disintegrations per minute (2) by the liters of air sampled (1), the number of disintegrations per minute per liter is obtained.
4. The working level is then determined by multiplying (3) by the working level factor (f) taken from Figure 3 of the text.

Summary

In estimating the relative hazards created by the inhalation of thoron and thoron daughters, certain assumptions were required because of incomplete knowledge of the biological behavior of these elements or were employed to simplify the calculations. These assumptions were: (a) All daughters produced by the decay of thoron in the lungs were retained in the lungs until they decay to stable lead; (b) The potential alpha energy of the daughters was calculated only from the energy released by the decay of ThB to stable lead; (c) Thorium A decayed 100 per cent of the time to thorium B; (d) Thorium C was considered to decay 33.2 per cent of the time by emission of a 6.07 Mev alpha particle; (e) Only the tissue dose delivered by the alpha particles was considered. Assumptions (b) through (d) were based on the radioactive properties of the nuclides, and introduced only small errors into the final result.

These calculations resulted in several interesting conclusions. They showed that ventilation is much more effective in reducing atmospheric concentrations of thoron daughters than it is for radon daughters. Conversely, practical rates of ventilation will not appreciably lower atmospheric concentrations of thoron. In comparison with radon daughters, much longer sampling times are feasible. This is important, as thoron daughter samples collected in the field will usually have quite small amounts of alpha activity and will require a more sensitive instrument than those which are satisfactory for radon daughter measurements.

The calculations indicate that a concentration of only eight micromicrocuries of thoron per liter at equilibrium with its daughters is equivalent in potential alpha energy to a concentration of 100 micromicrocuries of radon per liter at equilibrium (1.3×10^5 Mev/l). At equilibrium the calculated lung radiation dose from thoron is insignificant compared to the calculated lung radiation dose from thoron daughters. For example, a concentration of 5,360 micromicrocuries of thoron per liter delivers a lung radiation dose equivalent to that from a working level concentration of thoron daughters.

The calculations also indicate that, on an activity basis, the permissible atmospheric concentration of thoron daughters should be much less than the level for radon daughters. Therefore, all situations involving the processing and use of appreciable amounts of thorium ores, salts or metal should be surveyed to determine if significant amounts of these elements are present in the workroom atmosphere.

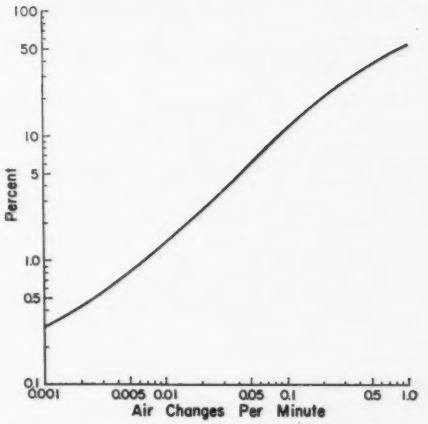

FIGURE 4. The percentage of the total dose contributed by thoron at various ventilation rates.

Acknowledgment

The authors wish to express their gratitude to R. L. Harris and R. E. Bales of the Occupational

Health Field Station, U. S. Public Health Service, for their advice and help in the experiments described in this paper.

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CUMMINGS AWARD

DR. GORDON C. HARROLD (left), recipient of the 1960 Cummings Memorial Award, accepts the certificate of award and congratulations from Elmer P. Wheeler, President of AIHA 1959–1960. The presentation was made at the Annual Banquet of the Association in Rochester, New York on April 27, 1960.

The Sub-acute and Chronic Toxicity of 1,1-Dimethylhydrazine Vapor

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Introduction and Background

UTILIZATION of 1,1-dimethylhydrazine (UDMH) in guided missile systems has promoted investigation of its potential health hazards. Previous studies have shown UDMH to be primarily a central nervous system stimulant causing convulsions and death irrespective of the route of administration.[1, 2] Oral dosages of 110 mg/kg caused hyperglycemia in anesthetized dogs.[3] McKennis *et al.* observed that 1,1-dimethylation resulted in decreased ability of the hydrazine moiety to cause fatty livers.[4] A study of the acute vapor toxicity of this compound has been reported by Jacobson, *et al.*[5] They described the material as a respiratory irritant and convulsant. Death occurred in two of three dogs after a 4-hour exposure to 111 ppm. (The third dog was sacrificed in view of impending death.) Rodents were somewhat less susceptible; the 4-hour LC_{50} for mice being 172 ppm, for rats 252 ppm, and for hamsters 392 ppm. Shook[6] cited an incident of human exposure that produced respiratory and gastrointestinal irritation. Investigators at the Medical College of Virginia reported that three exposures of dogs to 193 ppm of UDMH for 6 hrs/day caused convulsive seizures and mortality.[7] However, no toxic signs were observed in rats and guinea pigs after 14 such exposures.

Studies of longer duration are needed to estimate that quantity of toxicant to which man may be exposed without harmful effects. Therefore, these studies of 1,1-dimethylhydrazine vapor in rodents and dogs 6 hours/day, 5 days/week for periods of from six to 26 weeks were initiated.

Experimental

The liquid 1,1-dimethylhydrazine used in these experiments was obtained from Westvaco Chlor-Alkali Division. It was dispersed from bubblers by passing oil-pumped nitrogen through the material at flow rates necessary to achieve the vapor concentrations desired. After volatilization, the vapor was mixed with room air in a glass mixing-bowl and pulled into a 0.7-cubic-meter, dynamic gassing chamber operating at a flow of 0.15 M^3/min. Atmospheric samples were drawn from the chamber into collection bubblers containing 25 ml of distilled water and 1 ml of concentrated HCl. The bubbler contents were then analyzed for 1,1-dimethylhydrazine by an iodate-titration method.[8] An atmospheric concentration of 408 ppm of this vapor is equivalent to 1000 mg/M^3.

Male CWL (Wistar) rats weighing 200 gm, 20–30 gm female CF-1 mice, and male beagle dogs weighing approximately 11.4 kg were used in the various exposure sequences. Rodents were exposed to 140 ppm for six weeks and to 75 ppm for seven weeks. Dogs were exposed to 25 ppm for 13 weeks and to 5 ppm for 26 weeks. All animals were observed for toxic signs. Those surviving the experimental periods were killed and autopsied. Additional rodents were exposed and sacrificed throughout these experiments for study of macroscopic and microscopic changes in organs and tissues.

Dogs were bled from the radial vein at definite intervals throughout the exposure series for hematologic and clinical laboratory tests. Red and white blood cell counts and hematocrit determinations were carried out by methods described by Wintrobe.[9] Hemoglobin was determined by measurement of oxyhemoglobin of the blood using the Klett-Summerson photoelectric colorimeter.[10] The blood non-protein nitrogen was measured by a micro-Kjeldahl method[11] and blood sugar was estimated by the method of Folin and Malmros.[12] Bilirubin levels, including the total, direct, and indirect fractions, were determined by the method of Malloy and Evelyn combined with photoelectric colorimetry.[13] Sulfobromophthalein (BSP) retention was estimated by the intravenous injection of the dye in the amount of 5 mg/kg. A blood sample was withdrawn from another vein at the end of 20 minutes, the serum was alkalinized and the resulting color compared with a suitable standard.[10] The test is so arranged that a dog with normal liver function will show only 0–5% BSP retention in the blood after 20 minutes.

Results

Clinical Observations

Rats and mice exposed to concentrations of 75 and 140 ppm of UDMH showed occasional tremors during exposure. At the 140 ppm level, 29 of 30 mice and 1 of 20 rats died. The median survival time for the mice was three exposure days with a 19/20 confidence limit of 2–4 days as computed by the Litchfield Time-Per Cent Effect Method.[14] Rodents exposed at this concentration gained weight at a slower rate than the controls during the first two weeks of this experiment; thereafter weight gain was parallel to that of controls. At 75 ppm 8 of 20 mice died, while the only toxic signs observed in rats were periods of dyspnea and lethargy. Tonicoclonic convulsions were seen in all exposed rodents that died.

During the third day of exposure of three dogs to 25 ppm, the following toxic signs were noted: depression, salivation, emesis, diarrhea, ataxia (hind quarters), tonicoclonic convulsive seizures, injected oral and conjunctival membranes, bradycardia, fever, and death in one dog; similar signs without death in another dog; and depression and salivation only in the third dog. During this 13 week experiment the maximum mean weight lost in the two remaining dogs was 2.5 kg, while the maximum mean weight lost was 0.2 kg in three control dogs.

No severe toxic signs were observed in the three dogs exposed to 5 ppm, 6 hrs/day, 5 days/week for 26 weeks. After two to three weeks of exposure lethargy and some weight loss (without anorexia) became apparent. The exposed dogs had lost an average of 1.8 kg by the end of the 72nd exposure (14 weeks). Thereafter, the lethargic condition disappeared and the exposed animals regained some weight. At the conclusion of this exposure series the average weight loss of exposed dogs was 1.5 kg. The average weight loss in control dogs during this period was 0.7 kg. Exposure sequences are summarized in Table I.

Clinical Laboratory Tests

The results of clinical laboratory tests on dogs exposed repeatedly to 25 ppm of UDMH vapor are shown in Table II. Evidence of anemia after four weeks of exposure is indicated by decreases in hematocrit, hemoglobin and erythrocyte count. However, as the experiment continued hematocrit and hemoglobin approached pre-exposure levels, while the erythrocyte count remained somewhat lowered. Bilirubin levels, BSP retention, nonprotein nitrogen and blood sugar were found to be within normal ranges throughout the exposure.

The results of clinical laboratory tests on dogs exposed to 5 ppm of UDMH are shown in Table III. Mild anemia defined by decreases in hematocrit and hemoglobin was observed. Erythrocyte count remained within the normal range until the 24th week of exposure. Slight elevation in bilirubin became apparent later in the experiment and by the time exposures were terminated exceeded 2 mg%.

Pathology

Histology from rodents routinely sacrificed throughout these experiments revealed no morphological alterations due to 1,1-dimethylhydrazine vapor inhalation.

Examination of tissues of dogs surviving the 25 ppm experiment showed hemosiderosis in the cells of the reticuloendothelial system. Considerable pigment deposition was found in the Kupffer cells in the liver. The splenic sinuses contained

TABLE I

Summary of Animal Exposure Sequences to UDMH Vapor

Species	Number exposed	Concentration		Duration	Mortality
		ppm	mg/M³	wks	
Mice	30	140	342	6	29/30*
Rats	20	140	342	6	1/20**
Mice	30	75	183	7	8/30**
Rats	30	75	183	7	0/30
Dogs	3	25	61	13	1/3***
Dogs	3	5	12.2	26	0/3

* Deaths occurred within two weeks.
** Deaths occurred within five weeks.
*** Death occurred after third exposure.

TABLE II

Mean Clinical Laboratory Values for Two Dogs Exposed to 25 PPM of UDMH Vapor for 13 Weeks

Time of bleeding	RBC	Hbg	Hct
wk	10^6/mm³	gm %	%
Pre-exposure	9.81	*	50.8
4th	4.08	10.0	36.5
5th	5.14	10.0	38.5
7th	5.43	12.3	45.3
8th	5.98	12.4	49.3
9th	4.97	12.1	47.8
10th	5.77	12.5	48.0
12th	6.95	12.9	46.8
13th	5.94	12.7	45.0

* Pre-exposure determinations for hemoglobin in these experimental dogs were inadvertently omitted, however, male beagle hounds in this laboratory show a range of 15–17 gm% hemoglobin.

large amounts of heme pigment while less pigment deposition was noted in the lymph nodes and bone marrow. The bone marrow sections showed significantly increased activity of the erythrocytic series. Tissues of the dog that succumbed to this vapor showed alveolar hemorrhage, emphysema and atelectasis, but no hemosiderosis. Tissues of dogs exposed to 5 ppm showed hemosiderosis only in the spleen. Brain sections stained with hematoxylin-eosin and Weigert's myelin-sheath stain revealed no lesions. In addition, the following organs and tissues from the exposed animals showed no lesions: heart, kidneys, stomach, intestines, pancreas, adrenals, testes, bladder, and trachea.

Discussion

The studies reported herein have demonstrated that toxic effects may be produced in rodents and dogs by repeated exposure to low concentrations of 1,1-dimethylhydrazine vapor. Atmospheric concentrations of 140 ppm (342 mg/M³) and 25 ppm (61 mg/M³) in rodents and dogs, respectively, caused serious effects such as convulsions and death. Involuntary muscular contractions, both intermittent and tonic, were seen in all animals preceding death. In acute studies, Jacobson, *et al.* observed this finding in only mice.[5] Decreases in hematocrit, hemoglobin and red blood cell count defined further the toxic effects in dogs surviving 25 ppm of UDMH. Hemosiderin in the cells of the reticuloendothelial system of these dogs was most prominent in the liver; *i.e.* the parenchymal and the sinusoidal cells. The changes noted in the hematopoietic system and liver, viewed with clinical hematologic findings, demonstrate that 1,1-dimethylhydrazine at the 25 ppm level caused hemolytic anemia.

At 75 ppm (183 mg/M³) rats and mice showed periods of dyspnea and lethargy; the mice showed in addition, a 27 per cent mortality. Anemia and indirect bilirubinemia in the presence of mild lethargy without loss of appetite indicated hemolysis in dogs at the 5 ppm (12.2 mg/M³) level. Splenic hemosiderosis as well as anemia noted in dogs at this level was greatly reduced as compared to a similar finding in dogs at the higher exposure level. The order to decreasing species sensitivity to UDMH vapor is dogs, mice, and rats.

It seems likely in accidental human exposures to low concentrations of this vapor that lethargy and hemolytic anemia are the first toxic signs that would appear. Lethargy, a visible though nonspecific sign, may be more useful as a warning of impending health hazard than hematologic changes which require measurement.

TABLE III

Mean Clinical Laboratory Values for Three Dogs Exposed to 5 PPM of UDMH Vapor for 26 Weeks

Time of bleeding	RBC	Hbg	Hct	Bilirubin		
				Total	Direct	Indirect
wk	10^6/mm³	gm%	%	mg%	mg%	mg%
Pre-exposure...	7.19	15.4	52.9	0.2	0.0	0.2
2nd.....	7.43	15.1	53.3	0.2	0.1	0.1
6th.....	6.95	15.4	47.8	0.1	0.0	0.1
10th.....	7.25	12.5	45.3	—	—	—
14th.....	7.11	—	46.2	0.4	0.0	0.4
18th.....	—	14.0	47.3	—	—	—
24th.....	6.0	11.4	43.2	0.9	0.1	0.8
25th.....	—	—	—	0.4	0.0	0.4
26th.....	—	—	—	0.7	0.1	0.6
Post-exposure 2nd.....	—	13.6	46.0	2.5	0.2	2.3

Summary

Toxic effects seen in rodents from inhalation of 75 ppm and 140 ppm of 1,1-dimethylhydrazine are tremors, convulsions, and death. Dogs were significantly more sensitive; the resulting toxic effects from exposure to 25 ppm were hemolytic anemia, convulsive seizures, and death. Repeated exposure of dogs to 5 ppm for 26 weeks produced only mild toxic effects (slight lethargy, some hemolytic anemia, slight bilirubinemia). Based upon the results of these experiments there seems to be no doubt that for man the maximum allowable concentration (8 hrs/day, 5 days/week for prolonged periods) of 1,1-dimethylhydrazine vapor should be well below 5 ppm. Until more data and experience are available, it is suggested that 0.5 ppm be used as a guide to good industrial handling and safe practice.

Acknowledgment

We gratefully acknowledge the many helpful suggestions and guidance of Dr. Fred W. Oberst under whose supervision this study was conducted. Clinical laboratory tests for this study were performed by William Groff, U. S. Army Chemical Warfare Laboratories.

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Retiring Secretary

DR. WILLIAM G. FREDRICK (left) having completed a three-year term as Secretary of AIHA retired from that office. Below he is shown receiving a plaque in recognition of his services. Elmer P. Wheeler, retiring President, at the same time expressed the appreciation of the Association.

Measurement of Ketene for Study of Its Toxic Action on Animals

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THE ACUTE lethal effects of ketene on a variety of laboratory animals have been reported by Treon[1], Cameron and Neuberger[2], and Wooster, *et al.*[3] In addition experiments have been performed in this laboratory to test for the short-term toxicity of ketene for mice.

Long-term toxicity studies were indicated by the finding of Scott, *et al.* that ketene is formed by reaction of ozone with the olefins propylene and 2-pentene.[4] Validity of such studies depends upon the amount of objective knowledge of experimental conditions which delineate the studies. The purpose of this paper is to present an analysis of experimental variables involved in generation of and analysis for ketene during long-term studies of its toxicity for animals.

Apparatus

A suitable experimental apparatus for continuous generation of ketene was constructed for performance of the long-term studies (Figure 1). The entire apparatus was operated in a hood which was ventilated at 400 lineal feet/minute with a 0.76 ft² opening. The ketene mantle (A) was a near replicate of that described by Williams and Hurd.[5] Gases issuing from the mantle were passed through two efficient water-cooled condensers and into a vacuum-jacketed condenser (B) which was cooled by continuous circulation of alcohol. The alcohol was cooled by pumping it through the water-cooling coils of a modified drinking-water refrigerator (not shown in Figure 1) which was connected to the condenser with rubber tubing insulated with 4-inch diameter polystyrene foam tubing. Heat exchange in the refrigerator was accomplished by thermostatically controlled running water. The condenser maintained a mean temperature, measured in the thermometer well (C), of −33.3°C ±2.4°C measured over a period of 74 operating days with a total of 214 observations. The highest temperature recorded was −28.9°C, and the lowest −37.8°C. It served to condense most of the acetone which escaped from the first water-cooled condenser. Acetone collected in this condenser could be drained off through a stopcock at the bottom (D).

The gases, chiefly ketene and methane, issuing from the condenser at (E) could be by-passed into one of the evacuation ports for the hood at (F) or directed to the exposure chamber through a rotameter at (G). In practice, these gases were divided into a waste stream at (H), which comprised the greater volume (about 270 ml per minute), and the stream entering the exposure chamber (12 ml per minute). This stream of gases was mixed with air at (I) and passed at 400 ml per minute into a 100 cubic foot aluminum-painted aluminum exposure chamber with an aluminum-painted galvanized sheet-steel pyramidal top.[6] The gases from the ketene mantle mixed with air entered the exposure chamber at the top, were caught in a stream of air flowing at 78 cubic feet per minute, and were exhausted at the bottom of the chamber.

Output of Generator: Gases from the generator were led directly into two bubblers in series. The rate of flow of gases was held at a constant 288 ml per minute by means of a Cartesian Manostat inserted between the second bubbler and the vacuum source. The first bubbler contained 100 ml of hot 0.5900*N* NaOH and the second bubbler contained from 0.3 to one ml of hot alkali of the same normality, diluted with distilled water. For a series of ten measurements, the average time required to neutralize the alkali in the first bubbler was 11.22 ± 0.17 minutes, with a high and low value of 11.42 and 10.96 minutes respectively. The second trap was neutralized (to phenolphthalein) before the first when it contained 0.3 ml of the standard alkali, but after the first when it contained 0.35 ml, indicating an efficiency of absorption of more than 99.6 per cent by the first trap. Assuming all gases present to behave as ideal gases at the working conditions of 759.1 mm Hg pressure and 19.8°C, the molar value would be 24.1 liters. Considering then the first trap only, the mean volume of gas delivered was 288 × 11.22 = 3231 ml containing (0.059 × 24.1) 1000 = 1426 ml of ketene, which is 44.1 volume per cent of the total gases. If acetone pyrolyzed ideally to ketene and meth-

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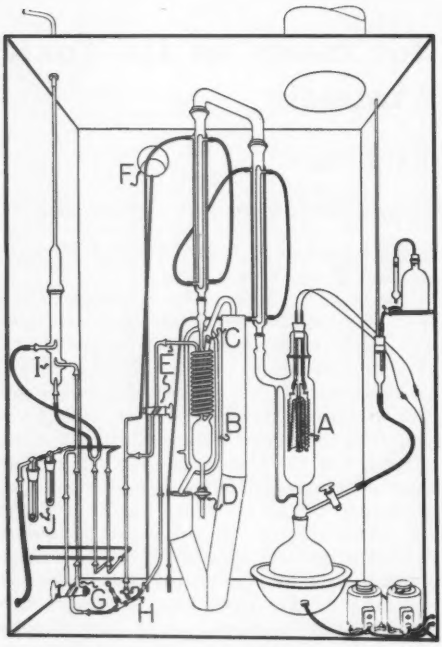


FIGURE 1. Apparatus for the generation, metering, and measurement of ketene. The front of the ventilated enclosure has been omitted for clarity.

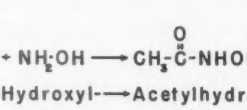
ane, the volume per cent of ketene would be 50, hence approximately 88.2% of the theoretical yield was achieved.

Method of Analysis

The method, essentially that of Diggle and Gage,[7] utilized the reaction of ketene with alkaline hydroxylamine to form hydroxamic acid which substance forms a colored complex with ferric ion.

$$CH_2{=}C{=}O + NH_2OH \longrightarrow CH_3{-}\overset{O}{\overset{\|}{C}}{-}NHOH \xrightarrow{Fe^{+++}}$$

Ketene + Hydroxylamine → Acetylhydroxylamine



Colored Complex

A pure grade of ethyl acetate was used as standard. Purity of the standard was tested by gas chromatography using 0.02 ml samples which were passed at 108°C through a ¼ inch × 12 ft copper tube containing C-22 (firebrick) coated with 30 gm/100 gm of Dow Corning high vacuum silicone grease. Flow rate of the helium carrier was 140 ml/minute. Other than an air peak, only one peak corresponding to ethyl acetate was observed. A stock standard was prepared by diluting 0.498 gm of ethyl acetate to 100 ml with anhydrous ethyl alcohol. The working standard was prepared as 1:100, 2:100 and 4:100 dilutions of the stock standard in anhydrous ethyl alcohol so that one ml aliquots would be equivalent to 0.5, 1.0 and 2.0 ppm of ketene, respectively in the exposure chamber using a 27.6 liter sample therefrom. This assumes the usual molar volume to be 24.5 liters and equivalence of ethyl acetate to ketene with respect to reaction with the hydroxylamine reagent, which is no doubt not exact, but is within a few per cent of being so.[7]

No major difficulties were experienced with the method for short-term studies when used as described by Diggle and Gage. Our use of the technique substituted ethyl alcohol for acetone as the solvent for the ethyl acetate standards, which greatly reduced reagent blanks. For long-term studies at low concentrations of ketene, the volume of reagents had to be doubled. Some difficulty was then experienced with duplication of results. This was traced to heating of the reaction mixture during addition of the $FeCl_3$ reagent which, when added in two minutes with vigorous stirring to the hydroxylamine solution, raised the temperature of the mixture to 63°C, dropping thereafter to 57°C in five minutes. The resulting reaction mixture appeared turbid. When the $FeCl_3$ was added at the same rate to the hydroxylamine cooled in an ice-water bath, the maximal temperature reached was 18°C and the reaction mixture remained clear.

$FeCl_3$ was added to the cooled hydroxylamine solution containing ethyl acetate equivalent to 1.0 ppm ketene. Separate tubes were then heated in a hot water bath to 50°, 60° and 70°C and plunged immediately into the ice-water bath and stirred vigorously. An unheated reagent blank was run. Color development was read after 30 minutes and plotted in Figure 2, uncorrected for turbidity.

A second experiment was run at the same ethyl acetate concentration, but the previously cooled tubes were heated to 60°C for 0, 0.5, 1.0, and 2.0 minutes after addition of the $FeCl_3$ reagent. The tubes were cooled immediately after heating. Color development is plotted in Figure 3, top curve.

Four blanks were prepared in the cold bath and heated to 60°C for 0, 0.5, 1.0, and 2.0 min-

utes. They were cooled with stirring as before. The absorbancies so obtained were subtracted from the corresponding values of the preceding run to obtain the values shown in the bottom curve of Figure 3. The difference between the two curves in this figure may be attributed to turbidity, whereas the bottom curve indicates the actual net color developed by the ferric hydroxamic acid complex.

When the reaction mixture was kept cool as described, the results were uniform within the boundaries set forth later in the statistical treatment. When deliberately heated, the results were not uniform and point to reactions inhibited at lower temperatures as the cause.

Selection of Analytic Procedure

Samples from the exposure chamber were passed through two traps in series, (J) in Figure 1, at the rate of 1460 ml per minute for 18.78 minutes. The value obtained from analysis of the second trap, which was added to that of the first, was small and variable and probably was due to aerosol carry-over from the first trap because simple passage of the sampled air over the surface of liquid in the first trap resulted in 75% trapping of the ketene assumed to be present. The remaining 25% was retained in the second trap which operated in the conventional manner. Since approximately two hours were required to complete each test for ketene under our conditions, a study was made to determine the simplest manner to carry out the analytic procedure commensurate with the degree of accuracy and precision inherent in the test method.

A series of 48 analyses was performed. Each analysis utilized a sample drawn from the exposure chamber. A reagent blank and three standards as described earlier corresponding to 0.5, 1.0, and 2.0 ppm were run concurrently with each unknown from the exposure chamber. All absorbancies were read against a distilled water blank using cuvets with a 10-cm light path in a Beckman D.U. Spectrophotometer at 540μ and a slit width 0.027 mm. The reading of the reagent blank was subtracted from the remaining readings to obtain the analytic number. After obtaining the 48 sets of analytic numbers, the following general methods were tested to select the method best suited to our purposes:

1. The formula for the linear regression line was calculated by the method of least squares for each of the 48 sets of determinations and the absorbance of the unknown was substituted therein to determine the concentration of ketene in ppm. This was considered the best possible way to determine the concentration of the unknown using three standards.

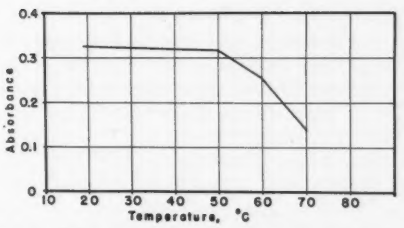


FIGURE 2. The effect of reaching the stated temperatures on the measurement of the ethyl acetate standard. Concentration of standard was held constant.

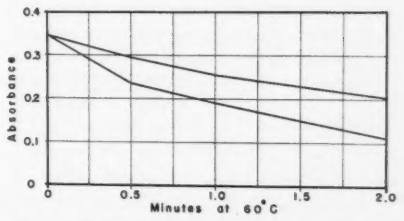


FIGURE 3. Upper Curve: The effect of heating to 60°C for the stated times on the measurement of the ethyl acetate standard, uncorrected for turbidity. Lower Curve: The upper curve has been corrected for turbidity giving actual yield of colored complex. The difference between the two curves measures turbidity caused by heating to 60°C.

2. Absorbancies of the three standards for each of the 48 experiments were plotted against the ppm represented by them. The visually best fitting straight line was drawn through these points and, with one exception, these lines were drawn arbitrarily through the origin. Concentrations of the unknowns were read from these lines at the abscissa.

3. The formula for the best fitting linear regression line was calculated by the method of least squares for the pooled sets of the 48 individual experiments. Each individual unknown was then calculated from the composite formula by substituting each individual absorbance of each unknown therein.

4. The value of each unknown was calculated from the formula:

$$\frac{\text{absorbance of unknown}}{\text{absorbance of standard}} \times \text{ppm in standard} = \text{ppm in unknown.}$$

TABLE I

Mean Differences and Standard Deviations of these Mean Differences from the Analysis for Ketene Obtained from Individually Computed Linear Regression Lines

	Range of ppm = 0.310 to 2.14		Range of ppm = 0.750 to 1.25	
Method	Mean difference from method 1	Standard deviation	Mean difference from method 1	Standard deviation
2	1.40%	1.23%	1.19%	1.12%
3	7.60	5.60	8.00	5.28
4	2.93	2.28	2.82	1.99

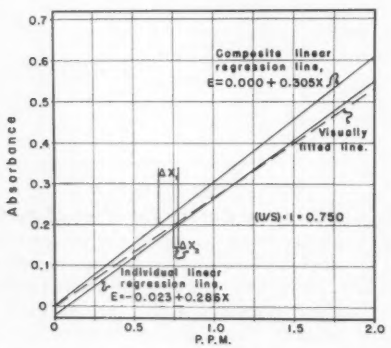


FIGURE 4. A comparison showing the difference between the value for a sample of ketene obtained by use of the composite linear regression line and the individually calculated linear regression line (Δx_1); between an individual visually fitted regression line and the corresponding calculated line (Δx_2). The difference between the value obtained for the sample using one standard and that obtained by substitution in the individually calculated regression line = Δx_2 in this sample.

The absorbance for the 1.0 ppm standard was used throughout since this was the central value expected for the unknowns.

The range of values for the unknowns was 0.31 to 2.14 ppm calculated from the individual least squares lines. Method 1 was used as the best possible value for each individual unknown. The difference between this value and the corresponding individual values obtained by methods 2, 3 and 4 gives a measure of their relative precision. These differences were calculated individually. The means thereof and their standard deviations are given in Table I, columns 2 and 3. A representative example is illustrated in Figure 4. The composite regression line is given and the three other methods for determining concentration are illustrated for one of the 48 determinations.

Because the values in actual exposure runs were expected to vary between 0.75 and 1.25 ppm, absorbance values corresponding to these concentrations were calculated from method 1. The values obtained were substituted in methods 2, 3, and 4 and the corresponding concentrations were calculated. The difference between the concentrations derived from methods 2, 3, and 4 and method 1 were calculated and are included in Table I, columns 4 and 5.

A statistical evaluation of the differences between the means for the broader range of concentrations was made. Method 3 was eliminated from consideration. Method 2 is significantly different from method 4 ($t = 4.077$, $P = 0.001$). Selecting a narrower range of concentrations did not improve the results significantly ($t = 0.8718$, $P = 0.4$ for comparison of the two ranges using method 2, and $t = 0.250$, $P = 0.8$ for comparison of the two ranges using method 4).

On the basis of these analyses, method 4 was selected to measure concentrations of ketene in the exposure chamber during the long-term studies. Reasons for this selection include savings in reagents, equipment, and time. These savings become important because even though the precision of method 4 is singificantly less than either method 1 or 2, it is at least as good as can ordinarily be obtained in photometric analysis of trace materials.

Linearity of Composite Regression Line

A complete analysis of variance, Table II, was performed on the composite regression line which was derived from the data compiled from

TABLE II

Analysis of Variance for the Composite Regression Line

Source	Sum of squares	d.f.	Mean square	F ratio
Between runs	0.159468	47	.003393	$F_1 = \frac{0.003393}{0.000247} = 13.7$
Between x-values combined regr.	5.193552	2 (1)	5.193552	$F_2 = \frac{5.193552}{0.000247} = 2,103$
Dev. from regr.	0.000252	(1)	0.000252	$F_3 = \frac{.000252}{.000247} = 1.02$
Regression × runs	0.054919	47	0.001168	$F_4 = \frac{.001168}{.00247} = 0.473$
Dev. × runs	0.011606	47	0.00247	
Total	5.419797	143		

the 48 experiments. This analysis subdivides the over-all variation in absorbance units into independent amounts of variability associated with differences between separate runs, between the three different concentration values (*i.e.*, variation due to regression) and differences in slopes from one run to another.

All tests were performed at the 1% level. The value for F_1 indicates a significant difference between runs with respect to the mean absorbance F_2 indicates a significant linear regression of absorbance on concentration; F_3 indicates that deviations from linearity of this regression are clearly not significant (the Lambert-Beer relationship is obeyed); finally, F_4 indicates that the slope of the straight line did not vary significantly from one experiment to another.

A discussion of the conditions affecting the use of hydroxylamine for the estimation of esters, anhydrides, lactones, etc., has been presented by Hestrin.[8] An examination of our results using this reagent reveals it to be a method of somewhat low accuracy but high precision. A single standard curve cannot be used for this reason, but when one known standard is included in the analysis, the results are well within acceptable limits.

Uniformity of Exposure Concentrations

Four analyses were performed on the chamber atmosphere with its full complement of animals and air flow of 78 cubic feet per minute, but without addition of ketene. These analyses failed to show a significant deviation between the tests and the reagent blank.

Although the placement of the animals in the chamber was changed daily, it seemed desirable to test for uniformity of concentration of ketene in the chamber. To do this, a series of analyses was performed on the concentration of ketene in the chamber until a decision was reached pertaining to the setting of the rotameter which would indicate a concentration of close to one ppm of ketene in the chamber. During actual exposure runs of the long-term experiment, chamber concentrations were estimated just above the animal cages at the center of each side of the chamber and at the center of the chamber. These analyses were performed three times daily at 10:00 A.M., and 1:00 and 3:00 P.M., until 33 values for each position were obtained. The weighted CT values (concentration in ppm × time in hours) for the high, low and mean measurements are given in Figure 5. Over a series of 200 measurements, the average concentration of ketene in the chamber was 1.02 ppm.

When the 165 sets of data for measurement of homogeneity of ketene in the chamber were secured, an analysis was performed to test for statistical normality of each of the five sets of populations and for equality of the five variances. Since both of these conditions were met at the 5% probability level, a test for equality of the five means was performed at the same probability level. Results of these analyses, summarized in Table III, indicate that the concentration of ketene in the exposure chamber was uniform at the five positions measured so that the concentration of ketene in the chamber could be estimated by sampling at any of these positions. In addition, the values varied uniformly which indicated the feasibility of performing one test daily. Therefore, after the

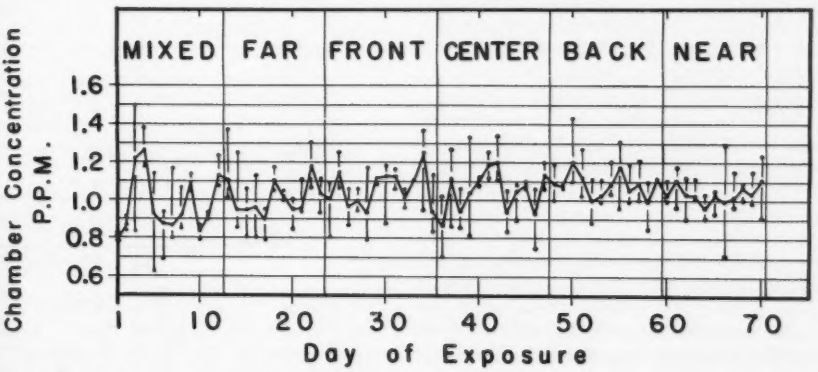


FIGURE 5. High, low, and weighted mean values for exposure chamber concentration of ketene. "Mixed" indicates random sampling points. "Far" and "Near" refer to sides of the exposure chamber with the ketene generator as reference point.

TABLE III
Statistics of Sampling Locations in Exposure Chamber

Statistic	Point of sampling				
	far	front	center	back	near
Mean (ppm)	1.02	1.06	1.04	1.08	1.04
Standard Dev.	0.14	0.13	0.16	0.13	0.11
High value	1.38	1.37	1.34	1.43	1.29
Low value	0.79	0.79	0.71	0.77	0.71

Analysis of variance

Source	Sum of squares	d.f.	Mean square	*F* ratio
Means	0.0542	4	0.01355	$\frac{0.01355}{0.01855} = 0.73$
within	2.9680	160	0.01855	
total	3.0222	164		$F_{.95}(4,160) = 2.43$

The five means of sampling location do not differ significantly at the 5% level of significance.

above analysis, chamber concentrations were performed at random times once daily to assess concentration of ketene in the exposure chamber.

Alteration of Ketene

Since gases from the ketene generator were delivered to the exposure chamber at the rate of 12 ml per minute, the flow of ketene to the chamber should have been $12 \times 0.441 = 5.29$ ml per minute. When mixed with 2.209×10^6 ml per minute of air this should have yeilded a mean concentration of 2.39 ppm of ketene in the exposure chamber. Since the actual value of 1.02 ppm was only 43% of this, studies were made on the rate of alteration of ketene using the Perkin-Elmer Infra-Red Spectrophotometer. One 10 cm cylindrical cell with two stopcocks and NaCl windows was used. This cell was kept in a desiccator over silica gel when not in use. Ketene and attendant gases were passed through the cell directly from the refrigerated condenser for 30 minutes for each sample, after which the pressure in the cell was reduced to 42 cm below atmospheric pressure in each case, and one cock opened to bring the pressure back to one atmosphere. The water content of the air was estimated from the wet and dry-bulb temperature of a psychrometer with assistance of a psychrometric chart.[9] Results of these experiments indicated that the only alteration of ketene measurable by this method was formation of the dimer, which was determined by initiation and increase of a peak at 5.37 micra. The amount of water vapor present in the air which was allowed to enter the absorption cell appeared to influence the rate of formation of the dimer. To test this further, the experiment was repeated using air which had passed through freshly oven-dried (120°C, overnight) silica gel. Careful attention was directed to the end that only air which had actually gone through the silica gel was allowed to enter the absorption cell, resulting in a diminution to almost zero in the rate of dimer formation.

Results of these measurements, given in Figure 6, show individual plots of absorbance vs time. The linear portions of the visually fitted individual curves were extrapolated to zero time and this value used as the "blank," was subtracted from each of the values on the corresponding curve. All curves appeared to be linear through the first 40 minutes. The increase in rate of formation of dimer did not appear to be a linear function of the concentration of water vapor, as illustrated in Figure 7 where absorbance of the dimer peak at 40 minutes is plotted against concentration of water vapor. This plot indicates the effect of water vapor on dimer formation to be exponential.

The possibility always exists that the order of a reaction might change with change in concentration of reactants, therefore, one cannot state categorically that half-lives measured at relatively high concentrations persist at concentrations near 1.0 ppm. Since the available equipment cannot measure ketene or its dimer at such low levels, this question was left unanswered. However, a number of facts tend to lend

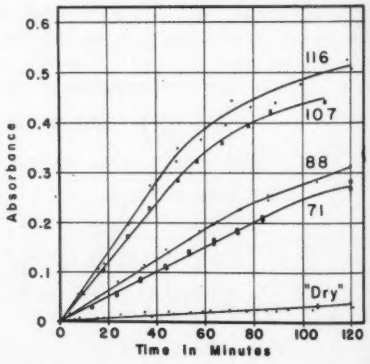


FIGURE 6. The effect of water vapor on formation and increase of ketene dimer. Absorbance measurements at 5.37 micra are plotted against time. The figures on the graph show grains of water per pound of dry air admitted to the infrared absorption cell. The line marked "Dry" was produced by forcing this air through dry silica gel.

strength to the assumption that atmospheric humidity played little, if any, part in the alteration of ketene in the exposure chamber. These are: (1) The time elapsed between first contact of ketene with the atmosphere and its ultimate evacuation from the exposure chamber was about 1.5 minutes. (b) The mean values of ketene concentration at the five different positions tested in the exposure chamber were the same. These sets of values were obtained serially over widely varying conditions of mean atmospheric humidity. (c) Ketene dimer, much less toxic than ketene[3] has been shown to be a β-lactone,[10] which have been shown to react with the ketene reagent.[5] (d) One molecule of dimer would react with hydroxylamine to yield a hydroxamic acid which should give approximately the same color with ferric ion as the acetylhydroxamic acid formed from ketene. (e) However, one molecule of dimer represents two molecules of ketene; hence one would anticipate an effect on the mean "ketene" concentration measurements if atmospheric humidity were a controlling factor. Thus, during the time which ketene was exposed to atmospheric humidity, the effect of dimerization was not sufficient to produce significant deviations in the mean concentration of ketene measured at various points in the chamber.

Finally, since most metals[11] are known to act catalytically with ketene, mercury vapor was used to test for this influence on rate of alteration of ketene. Ketene was collected in the usual way in the infrared cell which was then partially evacuated as before. The atmosphere which was permitted to enter the cell was saturated with mercury vapor from a partly filled, vigorously shaken bottle. Measurements which were repeated over a period of one hour for each sample revealed that the rate of alteration of ketene did not exceed that which was expected for dimer formation after consideration of the concentration of water vapor in the air.

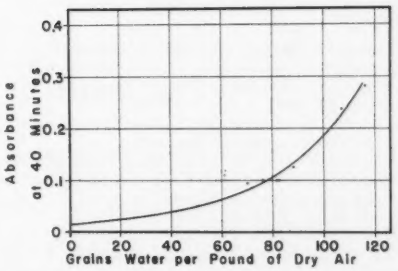


FIGURE 7. The effect of water vapor on formation and increase of ketene dimer. Absorbance values from Figure 6 at 40 minutes are plotted against water vapor permitted to enter the infrared absorption cell. The curve indicates an exponential effect of water vapor concentration on rate of formation of dimer.

Summary

1. An apparatus for generation and metering of ketene for long-term exposure studies is described.

2. The method of Diggle and Gage was found satisfactory for air analysis of high concentrations of ketene. For concentrations of ketene near one ppm the larger volume of reagents required must be cooled in ice-water to avoid variable results caused by heating.

3. Deviations from the Lambert-Beer Law were insignificant at the 1% level of probability for absorbancies from 0.0 to 0.6 with a light path of 10 cm.

4. A detailed study to determine the simplest manner to control the analysis revealed that a single standard curve should not be used; the equation of a standard curve of at least three points determined concurrently with each unknown would be best, but a single concurrently determined standard gives results within a few per cent of that obtainable from the equation of a concurrently determined 3-point standard curve.

5. Concentration of ketene in a 100 cubic foot exposure chamber was found to be uniform and reasons are given for adequateness of one test daily during long-term studies.

6. Approximately 43% of the ketene metered into the exposure chamber remained unaltered for measurement. Although moisture was found to affect exponentially the rate of conversion of ketene to its dimer, this effect was shown to be of no significance in these studies.

7. The presence of mercury vapor did not cause an increased rate of alteration of the infra-red spectrum of ketene within one hour.

Acknowledgments

The author is deeply grateful for the analysis of variance for the composite regression line which was performed by Dr. Eugene Harris of the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, and for the construction of the cooling unit and the chromatographic analysis of ethyl acetate by Mr. A. L. Mendenhall and Mr. Lial Brewer, respectively, both of the Occupational Health Program.

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PAST AND PRESENT

AS HIS FIRST OFFICIAL ACT on assuming office as President of AIHA 1960–61, Jack C. Radcliffe (right) presents a plaque to Past-President, Elmer P. Wheeler in recognition of his leadership and devotion to the interests of AIHA.

Data on Ultraviolet Absorption and Fluorescence Emission

J. A. HOUGHTON and GEORGE LEE

Loss Prevention Department, Liberty Mutual Insurance Company, Hopkinton, Massachusetts

THE USE of ultraviolet absorption for the quantitative determination of contaminants in air has been described for parathion,[1] benzene,[2] toluene[2] and acrylonitrile.[3] Fluorescence methods have been described for beryllium[4, 5, 6] and polynuclear aromatic compounds.[7] Additional data on absorption are available in the literature, which can be adapted to industrial hygiene procedures. We have attempted to tabulate available information obtained mainly from the literature, but supplemented by data from our own laboratory, in order to make it available to other industrial hygiene laboratories. Although there are many limitations to these methods, the ease, rapidity and sensitivity of determinations by ultraviolet absorption or fluorescence suggests their use wherever possible, for many tedious manipulations can be eliminated.

Reporting of Ultraviolet Data

The reporting of ultraviolet absorption data in the literature has not been consistent and it is difficult to compare one report with another. The ASTM Committee E-13 on Absorption Spectroscopy has proposed recommendations on the presentation of data[8] and we have followed one of the suggestions of this committee and we have drawn our curves plotting the logarithm of the molar absorptivity against the wavelength in millimicrons. Data presented in the literature by other methods has been converted to this same basis. The terms and symbols we have used are those defined in ASTM Designation E131[8] which are the following:

$$A = a\,b\,c = \frac{\epsilon}{M}\,b\,c$$

- A = absorbance (read directly on the spectrophotometer)
- a = absorptivity
- b = cell light path length in centimeters
- c = concentration in grams per liter (milligrams per milliliter)
- ϵ = molar absorptivity
- M = molecular weight.

Presented at the Twenty-first Annual Meeting of the American Industrial Hygiene Association, Rochester, New York, April 28, 1960.

The following columns are given in the tabulation of the UV data (see Table I): "MAC" is expressed in micrograms of contaminant per liter of air; "Log ϵ", is the logarithm to the base 10 of the molar absorptivity; "ϵ", and "a" are the molar absorptivity and the absorptivity with the relationship defined above. "c x 1000" is the concentration in micrograms per milliliter which would give an absorbance reading of 0.05 (about 90% transmittance) on the Beckman DU spectrophotometer. We have taken 0.05 as the lowest absorbance reading that it is desirable to use; other analysts prefer to use 0.10 as the lowest acceptable reading which would double the value of c x 1000 given in the tabulation.

All of the wavelengths given are maxima, but some are minor ones that are not the best suited for analysis; the minor peaks are given in parentheses and may be used to aid in identification of the material or can be used in some cases where other materials may interfere at the major peaks.

Reporting of Fluorescence Data

Only the major maxima have been tabulated (see Table II) in the fluorescence data and both the activation peaks and fluorescence peaks are given. There are no standards on reporting fluorescence intensities inasmuch as the instrumentation varies widely. The concentrations reported are the minimum values that are reliable, and values given in the literature have agreed closely with results in our own laboratory.

Reliability of Data

The maximum absorbance peaks for a particular material are not absolute and invariable, and the ultraviolet absorbance maxima reported from various sources may vary widely. The variations are caused by the differences in conditions of the tests and some of the causes are: (a) solvents used (polar or non-polar); (b) temperature; (c) types of excitation lamps, *e.g.*, mercury vapor vs. tungsten lamp; (d) slit width; (e) instrument calibration; and (f) pH value.

It is necessary for each laboratory to determine the peaks on its own instrument in order to obtain maximum sensitivity, and to record the conditions under which these peaks were obtained.

If the solvent and conditions indicated in the table are used, the difference should not be more than five millimicrons in the ultraviolet absorption, and is generally less. The extent of the differences can be seen with a few substances in the table where data are given from several sources. Also, with some fluorescent solutions, a strong UV absorbance maximum can be masked when the ultraviolet light is passed through the solution, for the fluorescence can affect the phototube and give a low absorbance reading. These masked peaks can be detected on a fluorimeter. In the tabulation, no attempt has been made to list the masked peaks, and only the apparent peaks are listed. A second fluorescent material in the solution can distort the spectra, as can some silica cuvettes which show traces of fluorescence.[9]

In the fluorescence maxima, the wavelengths found depend on the same factors as with the ultraviolet absorption readings, for theoretically the maximum fluorescence activation wavelength should also show as a maximum ultraviolet absorbance reading. But, in addition, the wavelengths depend to a considerable degree on the fluorimeter used, and there can be differences of as much as 15 to 20 millimicrons between reported data.

Some of the factors which affect fluorescence maxima were discussed by Hercules[10] and include the degree of overlap of the activation and fluorescence spectra, the concentration, the nonlinear response of the phototube and the effect of scattered light. Suspended dust or other solid particles in the solution can have a serious affect on the results and it is advisable to centrifuge solutions before making a fluorescence reading.

By following and maintaining standardized conditions, reproducible results can be obtained with quantitative fluorescence and ultraviolet absorbance methods. The data given in the tabulation will show the location of peaks and the magnitude of the absorbance at the peaks and will give the analyst a good start in setting up a method for sampling and analysis.

Use of UV and Fluorescence Procedures

Air sampling methods are assumed to be standard techniques that have been proven to be satisfactory and we are not attempting to discuss them. The procedures are most suitable where a single contaminant is present in the air, which we have found to be the case in actual practice for many of the substances listed, but many determinations can be made in the presence of other materials. Alcohols, aliphatic esters, aliphatic chlorinated hydrocarbons, aliphatic ketones and aliphatic hydrocarbons do not interfere in the amounts that will be found in most air samples because the absorption of these compounds in the ultraviolet range above 220 millimicrons is small in comparison with the compounds given in the table, and the fluorescence is negligible. Although the absorbance of these compounds is small it can be appreciable when they are used as the solvent and care must be used in the selection of the solvent. Fluorescence found in compounds of these classes generally come from impurities, for example USP ethanol will frequently show a fluorescence.

Where there are two materials present that show strong absorption in the ultraviolet, a determination of both materials is possible if there is a marked separation of the peaks, even though there is an overlapping of the curves. The method has been described by Hirt, *et al.*[11] and requires the determination of the absorbance (A) of the mixture at a selected wavelength and at the isoabsorptive point (this is where the absorption curves cross) and a knowledge of the absorptivity (a) of the pure compounds at the isoabsorptive point and at the selected analytical wavelength.

$$\frac{A_1}{A_2} = \frac{a_1 x + a_2 y}{a_3}$$

A_1 = absorbance determined on the mixture at a selected wavelength
A_2 = absorbance determined at the isoabsorptive point
a_1 = absorptivity of one of the components at the selected wavelength
a_2 = absorptivity of the second component at the selected wavelength
a_3 = absorptivity of the mixture at the isoabsorptive point
x and y are the relative proportions of the components.

A similar method for aromatic compounds was also described by Goldman[12] and both references show how the methods can be extended to three components.

It is difficult to make an ultraviolet determination on the Beckman DU spectrophotometer below 225 millimicrons. Grieger, *et al.*,[3] have shown how the spectrophotometer can be manipulated for the determination of acrylonitrile where the peak is about 204 mμ and the determination is made at 210 mμ. The procedure is somewhat complex and should be avoided if possible and peaks selected above 225 mμ, with the Beckman spectrophotometer.

Where there are two or more suitable peaks in the absorption curve, such as with aniline (Figure 1), the usefulness of the method can be ex-

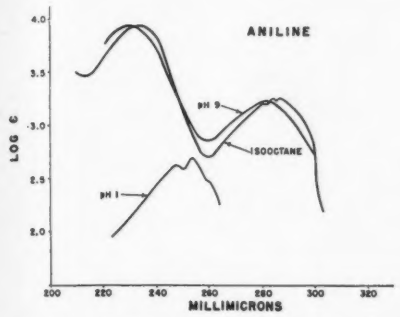

FIGURE 1. Aniline: spectrophotometric absorption curve showing several peaks suitable for analytic use.

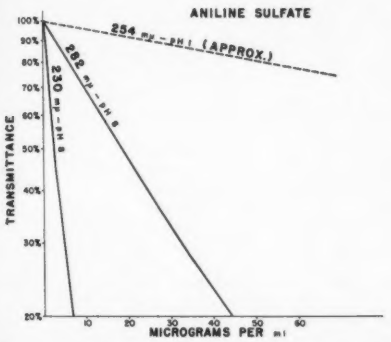

FIGURE 2. Aniline Sulfate: curves of concentration vs transmittance at three wavelengths.

tended. Aniline can be sampled in dilute sulfuric acid[13] and by making up to a known volume the solution can be read directly on the spectrophotometer at 254 mμ. If the concentration of aniline in solution is too low, the pH of the solution can be adjusted to about 8 to 9 and read at 282 mμ; if further sensitivity is needed, the same solution can be read at 230 mμ. Using these three wavelengths transmittance curves can be obtained as shown in Figure 2. It will be noted that there is a slight bend in the aniline curve where the UV absorbed is reemitted as fluorescent light which affects the phototube and apparently increases the transmittance. If greater sensitivity is required, then the same alkaline solution can be used for a fluorescence determination (Figure 3) activating at 280 mμ and reading the emission at 340 mμ. Between the fluorescence and ultraviolet methods for aniline concentrations ranging from 0.05 to several hundred micrograms per milliliter can be determined on the same solution.

Most of the substances listed in the table have a single maximum that is suitable for ultraviolet determination with the present instrumentation, but where two or more peaks are present it makes a convenient check possible where interfering substances may be present or to extend the range of concentrations that can be determined.

The ultraviolet absorption of iodine can be adapted to other analyses such as the determination of chlorine dioxide[13, 14] or bromine[15] where iodine is liberated from potassium iodide. The iodine potassium iodide complex shows a strong absorption in the ultraviolet and this sensitive method can replace the thiosulfate titration.

In the butanone-alkali method for toluene[13], the butanone extraction can be eliminated and the dinitrotoluene formed can be determined by UV absorption. It is necessary to salt out the potassium nitrate by the addition of isopropanol, because the nitrate ion has an appreciable absorption at the same wavelength.

Fluorescence methods are not as flexible as ultraviolet absorption, but the high sensitivity

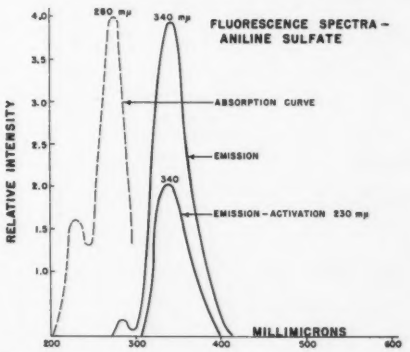

FIGURE 3. Fluorescence spectra of aniline sulfate.

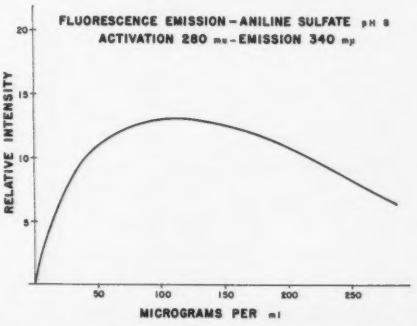

FIGURE 4. Fluorescence emission of aniline sulfate at pH 8: activation 280 mμ; emission 340 mμ.

TABLE I
Ultraviolet Absorption

Substance	MAC micrograms per liter of air	Maxima wavelength millimicrons	Log ε	ε	a	c × 1000 microgram per milliliter of solution	Solvent	Ref.
Acrylonitrile	45	202	3.66	4570			Water	10
		204	3.68	4760	90	0.6	Water	10
		(210)	3.15	1400	26	1.9	Water	10
Allyl benzene		(252)	2.30				Hexane	17
		260	2.35	220	1.9	26	Hexane	17
Aluminum-morin		265	4.40	25000	76	0.7	Water pH 4.6	18
		420	4.36	22700	69	0.7	Water pH 4.6	18
Aniline	19	234	3.93	8500	91	0.5	Isooctane	17
		287	3.25	1780	19	2.6	Isooctane	17
Aniline (sulfate)	19	248	2.62	420	4.5	11	Water pH 1	19
		254	2.68	480	2.7	18	Water pH 1	19
		230	3.93	8600	91	0.5	Water pH 9	19
		282	3.21	1620	17	3	Water pH 9	19
Anthracene		253	5.33	214000	1200	0.04	Cyclohexane	17
Benzene	80	244	2.00	100	1.3	39	Cyclohexane	17
		249	2.26	180	2.3	22	Cyclohexane	17
		255	2.35	224	2.9	17	Cyclohexane	17
		261	2.20	160			Cyclohexane	17
		(268)	0.98				Cyclohexane	17
		254.5					Isooctane	2
Benzidine	(.015)	283	4.40	25100	136	0.4	Ethanol	19
Benzoic acid		231	4.09	12300	100	0.5	Cyclohexane	17
		274	3.05	1120	9	5.5	Cyclohexane	17
		(282)	2.95	890	7	7	Cyclohexane	17
1,2-Benzpyrene		237	4.42	26000			Ethanol	17
		(267)	4.46				Ethanol	17
		(278)	4.55				Ethanol	17
		289	4.75	56200	223	0.2	Ethanol	17
		(304)	4.14				Ethanol	17
		(316)	4.35				Ethanol	17
		331	4.48	30000			Ethanol	17
3,4-Benzpyrene		225	4.42	26000			Ethanol	17
		(255)	4.60				Ethanol	17
		(266)	4.65				Ethanol	17
		(284)	4.67				Ethanol	17
		296	4.75	56200	220	0.2	Ethanol	17
		(347)	4.10				Ethanol	17
		363	4.36	23000			Ethanol	17
		384	4.45	28000			Ethanol	17
3,4-Benzpyrene-sulfonated		232					Water	19
		(259)					Water	19
		268					Water	19
		293					Water	19
		(305)					Water	19
		(365)					Water	19
		388					Water	19
Beryllium-morin	.002	280	4.34	22000	71	0.7	Water pH 11	18
		430	4.42	26100	84	0.6	Water pH 11	18
Boron-benzoin		370					Ethanol-basic	18

TABLE I—*Continued*

Substance	MAC micrograms per liter of air	Maxima wavelength millimicrons	Log ϵ	ϵ	a	$c \times 1000$ microgram per milliliter of solution	Solvent	Ref.
Chlorobenzene	350	210	3.87	7400	66	0.8	Water	22
		264	2.28	190	1.7	29	Water	22
Cholanthrene		220	4.57	37000	147	0.3		17
		(232)	4.53					17
		(262)	4.57					17
		(272)	4.57					17
		(284)	4.83					17
		295	4.87	74000	294	0.2		17
Chromate ion	0.1	275	3.57	3700	19	2.6		37
		370	3.68	4810	25	2.2		37
Chrysene		220	4.47	29500				17
		267	5.17	148000	650	0.08	Ethanol	17
		(294)	4.09					17
		(306)	4.11					17
		320	4.11	12900				17
o-Cresol	22	272	3.20	1580	14.6	3.4	Isooctane	23
		278	3.17	1500	14	3.5	Isooctane	23
		272	3.26	1800	17	3	Cyclohexane	17
		278	3.23	1700	16	3	Cyclohexane	17
		213	3.66	4560	42	1.2	Water	19
m-Cresol	22	273	3.16	1400	13	4	Isooctane	23
		280	3.20	1590	15	3	Isooctane	23
		273	3.21	1620	15	3	Cyclohexane	17
		279	3.21	1620	15	3	Cyclohexane	17
p-Cresol	22	264	2.85	710	6.5	8	Isooctane	23
		(273)	3.15				Isooctane	23
		(276)	3.21				Isooctane	23
		279	3.29	2000	19	2.5	Isooctane	23
		286	3.26	1800	17	3	Isooctane	23
		219	3.76	5750	53	1	Cyclohexane	17
		279	3.31	2040	19	2.6	Cyclohexane	17
		286	3.26	1820	17	3	Cyclohexane	17
1,2,5,6-Dibenzanthracene		221	4.82	66100	240	0.2	Ethanol	17
		(277)	4.75				Benzene	17
		(290)	4.96				Benzene	17
		300	5.11	129000	460	0.1	Benzene	17
		(322)	4.33				Benzene	17
		(336)	4.21				Benzene	17
		(351)	4.25	17800	64	0.8	Benzene	17
1,2,5,6-Dibenzanthracene-sulfonated		227					Water	19
		294					Water	19
		304					Water	19
		(326)					Water	19
		(342)					Water	19
		(358)					Water	19
3,4,8,9-Dibenzpyrene		260	4.72	52500			Ethanol	17
		(290)	4.55				Benzene	17
		(301)	4.95				Benzene	17
		313	5.22	166000	550	0.9	Benzene	17
		(380)	3.62				Benzene	17
		(400)	4.02				Benzene	17
		424	4.42	26300			Benzene	17
3,4,9,10-Dibenzpyrene		(317.5)					Reflectance	33
		(331.5)					Reflectance	33

TABLE I—*Continued*

Substance	MAC micrograms per liter of air	Maxima wavelength millimicrons	Log ε	ε	a	c × 1000 microgram per milliliter of solution	Solvent	Ref.
3,4,9,10-Dibenzpyrene (*Cont'd*)		(355)					Reflectance	33
		373					Reflectance	33
		394					Reflectance	33
Dibutyl phthalate		275	3.09	1240	4.5	1	Ethanol	24
p-Dichlorobenzene		224	4.09	12200	83	0.6		25
		(265)	2.47					25
		273	2.60	398	2.7	19		25
		281	2.51	324				25
DDT (p,p′)	1	237	4.27	18600	53	1	Ethanol	25
		(260)	2.84				Ethanol	25
		267	2.85	710	2	25	Ethanol	25
		(277)	2.62				Ethanol	25
		237	4.30	20000	57	1	Isooctane	25
		267	2.84	690			Isooctane	25
DDT (o,o′)	1	233	3.90	7900	22	2.3	Ethanol	25
		271	2.87	740			Ethanol	25
		233	4.03	11000	31	1.6	Isooctane	25
		270	2.95	890	2.5	20	Isooctane	25
DDT (o,p′)	1	236	4.16	14400	41	1	Ethanol	25
		269	2.83	680			Ethanol	25
		277	2.69	490			Ethanol	25
		236	4.18	15000	42	1	Isooctane	25
		269	2.83	680			Isooctane	25
Diethyl phthalate		275	3.08	1210	5.5	0.9	Ethanol	24
N-Dimethyl aniline	25	250	4.13	13500	110	0.5	n-Heptane	17
		293	3.36	2290	19	2.6	n-Heptane	17
2,4-Dinitrophenol	(1)	253	4.01	10200	55	1	Ethanol	24
		292	3.96	9200	50	1	Ethanol	24
2,4-Dinitrotoluene	1.5	240	4.15	14280	78	0.6	Ethanol	24
		248	4.12	13100	72	0.7	Ethanol-60% Water	19
Diphenyl		246	4.23	17000	110	0.5	Cyclohexane	17
Diphenyl methane		260	2.70	500	3	17		17
		(268)	2.60					17
Ethyl benzene	870	262	2.35	220	2	24	Isooctane	35
		(265)	2.23				Isooctane	35
		(268)	2.28	190	1.8	28	Isooctane	35
Fluoranthene		235	4.57	37000	183	0.3	Ethanol	17
		(276)	4.35				Ethanol	17
		286	4.50	31600			Ethanol	17
		(302)	3.54				Ethanol	17
		(322)	3.75				Ethanol	17
		342	3.88	7590			Ethanol	17
		357	3.91	8130			Ethanol	17
		287.5					Reflectance	33
		(323)					Reflectance	33
		(344)					Reflectance	33
		359					Reflectance	33
Iodine	1	289	4.58	37800	150	0.3	Water 5% KI	29
		352	4.41	25500	101	0.5	Water 5% KI	29
		311	3.92	8240	32	1.5	Toluene	29

TABLE I—*Continued*

Substance	MAC micrograms per liter of air	Maxima wavelength millimicrons	Log ε	ε	a	c × 1000 micrograms per milliliter of solution	Solvent	Ref.
Lithium-oxine		245					Ethanol-basic	18
		255					Ethanol-basic	18
		335					Ethanol-basic	18
		370					Ethanol-basic	18
Methoxychlor	15	229	4.18	15300	44	1	Ethanol	25
		(237)	4.12				Ethanol	25
		(245)	4.10				Ethanol	25
		273	3.49	3090	9	5.5	Ethanol	25
9 Methyl anthracene		256	5.27	186000	970	0.05	Ethanol	17
		(332)	3.43				Ethanol	17
		(348)	3.75				Ethanol	17
		366	3.94	8710			Ethanol	17
		386	3.92	8320			Ethanol	17
20-Methylcholanthrene		222	4.53	34000	130	0.4		17
		235	4.50	31600				17
		(261)	4.57					17
		(274)	4.61					17
		(285)	4.87					17
		297	4.95	89000	330	0.15		17
Methyl parathion	(0.1)	215	3.95	8910			Ethanol	25
		273	3.97	9390	36	1.4	Ethanol	25
Methyl styrene	480	245	4.07	11800	100	0.5	Ethanol	17
		285	3.07	1180	10	5	Ethanol	17
		297	2.90	790	6.7	7.5	Ethanol	17
Naphthalene		220	5.00	100000	780	0.06	Ethanol	17
		(266)	3.72				Ethanol	17
		276	3.75	5620	44	1	Ethanol	17
		(286)	3.60				Ethanol	17
		(311)	2.38				Ethanol	17
α-Naphthylamine	(.01)	240	4.15	14000	98	0.5	Water	19
		305	3.58	3800	27	2	Water	19
		239	4.35	22400	157	0.3	Ethanol	17
		322	3.71	5130	36	1.4	Ethanol	17
β-Naphthylamine	(.001)	237	4.80	63100	440	0.1	Ethanol	17
		282	3.82	6611	46	1	Ethanol	17
		338	3.30	2000			Ethanol	17
Nicotine	0.5	262	3.44	2740	17	3	Ethanol	25
o-Nitroaniline		231	4.22	16600	120	0.4	Ethanol	24
		276	3.67	4900	36	1.4	Ethanol	24
		403	3.72	5250			Ethanol	24
m-Nitroaniline		235	4.20	15900	115	0.5	Ethanol	24
		373	3.18	1500	11	4.5	Ethanol	24
p-Nitroaniline	6	228	3.80	6300	45	1	Ethanol	24
		371	4.18	15200	110	0.5	Ethanol	24
Nitrobenzene	5	256	3.89	7670	62	0.8	2-Ethyl hexanol	11
o Nitrophenol		272	3.78	6000	43	1.2	Ethanol	24
		346	3.50	3140	23	2.2	Ethanol	24
		277	3.80	6350	46	1.1	pH 5.2	11
		349	3.48	3020	22	2.3	pH 5.2	11

TABLE I—*Continued*

Substance	MAC micrograms per liter of air	Maxima wavelength millimicrons	Log ε	ε	a	c × 1000 microgram per milliliter of solution	Solvent	Ref.
m-Nitrophenol		230	3.98	9600	69	0.7	Ethanol	24
		270	3.75	5600	40	1.2	Ethanol	24
		332	3.32	2090			Ethanol	24
p-Nitrophenol		225	3.85	7100	51	1	Ethanol-acidic	24
		312	4.03	10600	76	0.7	Ethanol-acidic	24
		234	3.74	5500	40	1.2	Ethanol-basic	24
		402	4.28	19000	136	0.4	Ethanol-basic	24
		315	4.02	10450	75	0.7	pH 5.2	11
		426	4.44	27600	200	0.25	Dimethylformamide-basic	30
		415	4.34	22000			Dimethylformamide-25% H_2O	30
		410	4.30	20000			Dimethylformamide-50% H_2O	30
		406	4.26	18000			Dimethylformamide-75% H_2O	30
		401	4.22	16600	120	0.4	Dimethylformamide-95% H_2O	30
o-Nitrotoluene	30	257	3.73	5400	40	1.2	Ethanol	24
p-Nitrotoluene	30	215	3.88	7590			Ethanol	24
		273	3.98	9500	70	0.8	Ethanol	24
Parathion	0.1	274	4.00	10000	34	1.5	Ethanol	1
Pentachlorophenol	0.5	224	4.38	24200	91	0.6	Isopropanol	19
		258	3.94	8770	33	1.5	Isopropanol	19
		328	3.62	4200	16	3	Isopropanol	19
Perylene		251	4.64	44000	175	0.3	Ethanol	17
		(386)	4.08				Ethanol	17
		(407)	4.43				Ethanol	17
		434	4.53	34000			Ethanol	17
		(390)					Reflectance	33
		409					Reflectance	33
		(431)					Reflectance	33
Phenanthrene		(223)	4.36				Cyclohexane	17
		252	4.81	64600	363	0.1	Cyclohexane	17
		(275)	4.16				Cyclohexane	17
		(281)	4.04				Cyclohexane	17
		293	4.18	15100	85	0.6	Cyclohexane	17
Phenol	19	(265)	3.14				Cyclohexnae	17
		271	3.31	2040	22	2.5	Cyclohexane	17
		278	3.25	1800			Cyclohexane	17
		275	3.25	1800	19	2.6	Dimethylformamide	30
		281	3.18	1500			Dimethylformamide	30
		310	3.30	2000	21	2.4	Dimethylformamide-basic	30
		280	3.30	2000			Ethanol-basic	30
		290	3.31	2030	22	2.5	Ethanol-basic	30
		271	3.18	1500	16	3.1	Water pH 1 to 5	19
Pyrene		(231)	4.65				Ethanol	17
		241	4.94	87000	430	0.1	Ethanol	17
		(262)	4.43				Ethanol	17
		272	4.69	49000			Ethanol	17
		(305)	4.70				Ethanol	17
		334	4.67	46800	230	0.2	Ethanol	17
Pyridine	30	(252)	3.39				Ethanol	17
		257	3.44	2750	35	1.4	Ethanol	17
		(262)	3.28				Ethanol	17

TABLE I—*Concluded*

Substance	MAC micrograms per liter of air	Maxima wavelength millimicrons	Log ϵ	ϵ	a	$c \times 1000$ microgram per milliliter of solution	Solvent	Ref.
Pyridine (*Cont'd*)		255	3.74	5500	70	0.7	Ethanol-HCl	17
		256	3.73	5360	68	0.7	Water-acidic	19
		257	3.44	2750	35	1.4	Water-pH7	19
Styrene	420	251	4.13	13500	130	0.4		31
		(282)	2.95					31
		(291)	2.76					31
		249	4.06	11500	100	0.5	Ethanol	19
m-Terphenyl		250	4.65	44700	190	0.3	Chloroform	17
p-Terphenyl		280	4.40	25100	110	0.5	Chloroform	17
Tetralin		267	2.75	562	4	12	Isooctane	17
		274	2.75	562	4	12	Isooctane	17
Tetryl	1.5	225	4.40	25000	87	0.6	Ethanol	24
Toluene	750	(255)	2.30	200	2.2	23	Cyclohexane	17
		262	2.45	282	3	16	Cyclohexane	17
		269	2.39	246	2.7	18	Cyclohexane	17
		268					Iosoctane	2
o-Toluidine	22	234	3.95	8910	83	0.6	Isooctane	17
		287	3.32	2090	20	2.5	Isooctane	17
m-Toluidine		236	3.95	8910	83	0.6	Isooctane	17
		288	3.27	1860	17	3	Isooctane	17
2,4,6-Trinitrophenol		(292)	3.88				Ethanol-acidic	24
		335	3.73	5400	23	2	Ethanol-acidic	24
		358	4.15	14100	61	0.8	Ethanol-basic	24
Trinitrotoluene	1.5	227	4.29	19700	87	0.6	Ethanol	24
o-Xylene	870	263	2.41	259	2.4	21	Isooctane	35, 32
		271	2.33	212	2	25	Isooctane	35, 32
m-Xylene	870	(255)	2.22				Isooctane	35, 32
		(260)	2.30				Isooctane	35, 32
		265	2.44	278	2.6	19	Isooctane	35, 32
		(269)	2.33				Isooctane	35, 32
		273	2.43	269			Isooctane	35, 32
p-Xylene	870	(260)					Isooctane	35, 32
		(266)					Isooctane	35, 32
		268	2.72	524	5	10	Isooctane	35, 32
		274	2.79	625	6	8	Isooctane	35, 32
Zirconium-flavonol		245					0.2*N* H_2SO_4	18
		345					0.2*N* H_2SO_4	18

Table II

Fluorescence

Substance	Activation wavelength millimicrons	Emission wavelength millimicrons	c × 1000 micrograms per milliliter of solution	Solvent	Ref.
Aluminum-morin	430	500	0.0005	Water pH 4.6	18, 20
Aniline	290	360		Water pH 9	20, 36
	280	340	0.07	Water-basic	21, 19
	(230)	340		Water-basic	19
1,2-Benzanthracene		409		Reflectance	32
Benzidine	285	375	0.008	Isooctane	19
	285	395		Ethanol	19
Benzoic acid	— NONE —			Water	21
1,12-Benzperylene		399			34
		408			34
		420			34
		430			34
	305	430			21
	375	430			21
	395	430			21
1,2-Benzpyrene	290	410		Cyclohexane	21
	330	410		Cyclohexane	21
3,4-Benzpyrene		405.5			32
		428			32
		405		Reflectance	32
		429		Reflectance	32
		(456)		Reflectance	32
	375	397			27
	375	418			27
3,4-Benzpyrene-sulfonated	260	425		Water	19
	295	425		Water	19
	375	397		Water	19
	375	418		Water	19
Beryllium-morin	470	590	0.004	Water pH 11	18, 20
	275	530		Water-basic	19
	430	530		Water-basic	19
Boron-benzoin	370	480	0.2	Ethanol-basic	18, 20
Carbon tetrachloride	— NONE —				19
Chrysene	250	360			21
	300	380			21
	310				21
m-Cresol	280	305		Water-acidic	21
	280	310	0.4	Water-pH 1 to 4	19
o-Cresol	275	305		Water-acidic	21
	270	300	0.4	Water-acidic	19
p-Cresol	285	315		Water-acidic	21
Cresol (commercial mixture)	280	310	0.4	Water pH 4	19
1,2,5,6-Dibenzanthracene-sulfonated	305	400		Water	19
3,4,8,9-Dibenzpyrene	310	480			21
	335	510			21
	390				
	410				

TABLE II—*Continued*

Substance	Activation wavelength millimicrons	Emission wavelength millimicrons	c × 1000 micrograms per milliliter of solution	Solvent	Ref.
3,4,9,10-Dibenzpyrene	255	455			21
	290	485			21
	300				21
	395				21
	415				21
DDT (p,p′)	— NONE —			Methanol	26
DDT (o,o′)	— NONE —			Methanol	26
DDT (o,p′)	— NONE —			Methanol	26
Diphenyl	260	310	0.1	Isopropanol	19
Fluoranthene	364	465			27
	293	465			27
	360	460		Reflectance	28
	235	470		Reflectance	28
	(285)	470		Reflectance	28
Lindane	— NONE —				26
Lithium-oxine	370	580	3	Ethanol-basic	18, 20
Methoxychlor	— NONE —			Methanol	26
9-Methyl anthracene	250	500		Reflectance	28
20-Methylcholanthrene	230	460		Reflectance	28
	300	400		Reflectance	28
	300	420		Reflectance	28
	(365)	(420)		Reflectance	28
	(365)	(460)		Reflectance	28
Naphthalene (sulfonated)	282	330		Water	19
	282	(650)		Water	19
	282	(665)		Water	19
α-Naphthylamine	235	445	0.004	Water	19
	310	445		Water	19
p-Nitroaniline	— NONE —			Water	19
Nitrobenzene	— NONE —			Isooctane	19
Oil mist (sulfurized fats in petroleum base)	350	450	9	Carbon tetrachloride	19
Oil mist (unknown type)	280	340	20	Carbon tetrachloride	19
Oil mist (petroleum base)	370	460	1	Carbon tetrachloride	19
Perylene	240	475		Reflectance	28
Phenol	270	310		Water pH 1	21
Pyrene	340	390			27
Pyridine	— NONE —			Water	19
m-Terphenyl	270	340	0.1	Chloroform	19
p-Terphenyl	280	340	0.1	Chloroform	19
Warfarin	320	385	0.2	Methanol	26
Zirconium-flavonol	400	465	0.1	0.2*N* H_2SO_4	18, 20

Note: Oil mists from cutting and lubricating oils can be quantitated from fluorescence characteristics. The maximum activation and emission wavelengths must be determined on each individual mixture, and the three examples given show the wide variations that may be expected. "Reflectance" data was obtained from chromatogram spot on filter paper.

makes use desirable in many instances. Fluorescence generally requires a comparatively pure material for an accurate determination for there can be interferences either by quenching of the fluorescence or masking by another fluorescing material.

The activation and emission peaks of the fluorescing material can shift with some materials with changes in concentration, for example, quinine in sulfuric acid shows a fluorescence maximum at 450 mμ in concentrations varying from 10 to 0.005 micrograms per ml, but at 0.001 micrograms per ml the maximum is at 410 mμ.[16] We have observed a similar shift with p-terphenyl with changes in concentration. With increasing concentration there will be self absorption of the light emitted as shown in Figure 4. If there is a possibility of an unusually high concentration, this can be detected by diluting the solution and there will be a higher reading if the concentration is on the wrong side of the curve.

The tabulation of such data (see Tables I and II) can be useful in estimating how much air to sample knowing the minimum concentration, c, in solution and the concentration in air at the MAC. If the air sample is in d milliliters of solution and the air to be sampled is L liters, then:

$$\frac{c(\text{micrograms per milliliter}) \times d(\text{milliliters})}{\text{MAC (micrograms per liter)}} = L \text{ (liters of air)}$$

With this amount of air it would be possible to determine concentrations in air down to the MAC; if it was desired to determine down to 0.1 of the MAC then 10 times this volume of air should be sampled.

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Determination of Decaborane and Pentaborane by Means of Triphenyltetrazolium Chloride

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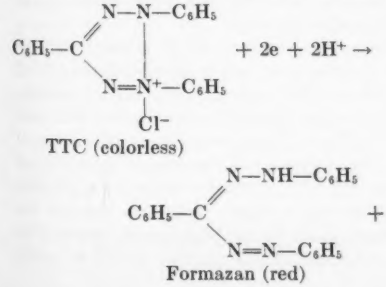
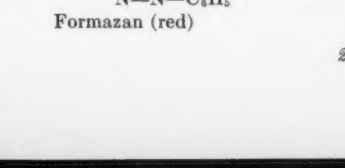
DURING the past several years a number of methods have been published which concern themselves with the microanalysis of borohydrides, and a reasonably complete reference list to these efforts has been given in a paper by Hill et al.[1] Very surprisingly there is only little information on the analysis of pentaborane among these papers, and in particular there has not been described a single method for a colorimetric determination of this important borane. An instrumental method published by Kuhns[2] makes use of a color spot test, and it is based on an observation made by the senior author (W.H.H.) a number of years ago[3] that the well-known reagent triphenyltetrazolium chloride gives a red color with decaborane as well as with pentaborane. This finding established for the first time that pentaborane could be determined colorimetrically, and it is the object of this paper to relate work done in this field in the course of the years.

The first experimental work with this reagent was carried out with decaborane, and it was found that an alkaline solution of triphenyltetrazolium chloride (hereafter referred to as TTC) is reduced by decaborane to the corresponding water insoluble formazan which is soluble with red color in a number of solvents, such as xylene and dioxane. The reaction does not occur in neutral or acid solution. The following equation is believed to depict the reaction involved:



TTC (colorless)



Formazan (red)

The color intensity of the red solutions thus obtained appeared in qualitative tests to bear a relationship to the amount of decaborane employed, which indicated that a quantitative colorimetric method of analysis for boranes might well be developed on this basis.

The applicability of this principle to pentaborane analysis was established with a current of nitrogen containing 56.5 ppm of pentaborane. When it was passed through two Vigreaux-type bubblers containing an alkaline solution of TTC and xylene, using a rate of 0.4–0.6 liter per minute, the xylene in the first bubbler became colored red while no color appeared in the second bubbler for a considerable period of time. Thus it was proven that pentaborane does react with TTC, and that the reagent-solvent mixture is an effective retaining agent for pentaborane.

This finding was further verified, and some quantitative relationship established, in connection with animal inhalation tests in progress at the time with chamber air containing about eight ppm of pentaborane. Three Vigreaux bubblers were used for a test. Each bubbler contained 2 ml of 0.1% aqueous TTC, 5 ml of 0.6*N* NaOH (normality not critical), and 20 ml of xylene, and the chamber air was passed through them for definite periods of time. The contents of the bubblers were then combined in a separatory funnel, shaken thoroughly, and the colored xylene layer separated from the aqueous phase. Spectrophotometer readings of the color intensity were made at a wavelength of 490 millimicrons at which maximum absorption occurs for

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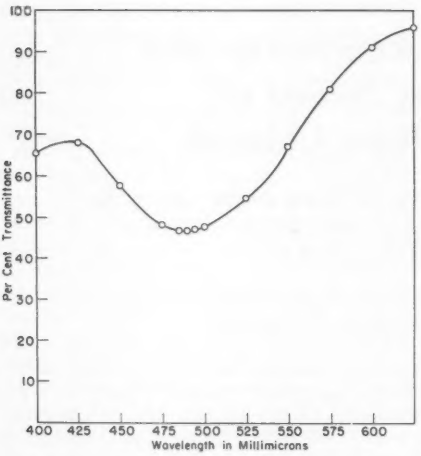

FIGURE 1. Spectrophotometric absorption curve for formazan derived from triphenyltetrazolium chloride.

this compound (see Figure 1). The values obtained in this manner showed that there was general correlation of color with gas volume, and thus with the amount of pentaborane. Fluctuations in the pentaborane level of the chamber air and other factors, not then apparent, were probably responsible for observed deviations from a straight line relationship. The TTC reagent was found useful even at much lower pentaborane concentrations, namely 0.2 ppm or less: a 30 liter sample of the chamber air passed through a mixture of xylene, aqueous TTC and aqueous sodium hydroxide produced an orange-red xylene layer.

The fact that the reaction of TTC with boranes occurs best in an alkaline aqueous medium, while the product of the reaction, the formazan, must be dissolved in an organic solvent to display its red color, suggested from the beginning of this investigation that two reagent systems were possible: (a) a single phase system containing the TTC, the alkali, water, and the organic solvent; and (b) a two-phase system composed of an aqueous and an organic layer with the respective components dissolved in the two phases. The latter was used in the first tests made in this field, but it was realized from the beginning that a single phase system would have tremendous advantages.

Dioxane was considered to offer an excellent chance for developing a single phase system because of its complete miscibility with water and its great solvent power for many organic materials. Uniform red solutions were indeed easily produced from decaborane, alkali, TTC and dioxane, the latter functioning as the mutual solvent. Unfortunately dioxane itself gave a fair amount of red color when mixed with aqueous TTC, so that the blank in this method would be considerable and perhaps prohibitive, if variable. On the assumption that the color producing impurity in the dioxane could be a peroxide, the solvent was distilled by itself and after treatment with potassium iodide. The blank color was thus improved very considerably, but it was not eliminated. This does, of course, not mean that further work with dioxane might not establish it as a useful mutual solvent for the TTC method, but its use was abandoned for the time being.

Other solvents tried and found wanting for one reason or another were methyl-, ethyl-, and isopropyl alcohol, acetone, methylisobutyl ketone, diisobutyl ketone, dibutyl ether, chloroform, and cyclohexane. The idea of using an amine as an organic "alkali" in combination with xylene or similar solvent was tried out with n-amylamine and with tri-n-butylamine. The former gave too much "blank" color with TTC, but the latter did not, while both produced (mixed with xylene) well colored solutions with TTC and decaborane. Mixtures of xylene, methanol and tri-n-butylamine also proved of some value, with very little blank color and excellent color with decaborane (and TTC).

These amines are expensive and the systems built on them were not considered seriously until Kuhns,[2] one of the authors, used pyridine for the purpose. While its odor may not be any more pleasing to the nostrils of laboratory workers than that of other amines, at least it has the advantage of being relatively very cheap and easily available in large quantities. It shows no blank color with TTC, even after hours, it is an excellent general solvent, and it gives fine color with boranes and TTC. Single phase systems based on pyridine as a mutual solvent therefore became the most commonly used ones in this investigation, though it will be shown that two-phase systems are entirely feasible and can be utilized by those faint of heart who do not want to employ pyridine.

As far as the reagent itself is concerned, a number of analogues and homologues were tried out: neotetrazolium phosphate, neotetrazolium chloride, tetrazolium red, tetrazolium violet, and blue tetrazolium. They seemed to offer no particular advantages for the problems at hand and their use was not pursued further, though blue tetrazolium was actually tried on animal exposure chamber air containing pentaborane. The intense color of the reagent itself in xylene made

the evaluation of additional color produced by the borane illusory. However, this field bears additional, and more detailed, investigation because some interesting observations were made, such as a better extraction of color in a two-phase system utilizing neotetrazolium phosphate, with good color differentiation between sample and blank, and the fact that blue tetrazolium seemed to give the strongest color of them all with pentaborane.

The construction of a dependable calibration curve proved very difficult for a number of reasons. Pentaborane is a very volatile liquid (b.p. 58°C) which, moreover, is apt to explode spontaneously in contact with air. It is, therefore, not possible to weigh it out directly, at least not under ordinarily prevailing laboratory conditions. Because of its ease of hydrolysis with water in liquid or vapor form, and then existing lack of methods of analysis, the pentaborane content of the air of the exposure chambers could not be ascertained with an accuracy needed for such standardization. Recourse was had, therefore, to a solution of pentaborane in xylene which was furnished by the Callery Chemical Company. The solution was kept in a nitrogen pressured steel cylinder in order to avoid degradation as far as possible. Even so, spontaneous decomposition with the formation of other boron hydrides and/or hydrogen, or reaction with the hydrocarbon employed as the solvent, over longer periods of time, could not be ruled out entirely because such rearrangements and reactions are known from other boranes, such as diborane.

The pentaborane solution furnished by Callery Chemical Company repeatedly contained 0.634 grams of pentaborane per liter of solvent, or 543 micrograms of boron per ml. This figure was verified with a sufficiently close tolerance by hydrolyzing a freshly drawn sample with 1*N* sodium hydroxide and subsequent methyl borate distillation, which yielded figures of about 530 micrograms of boron per ml of xylene solution. Other methods of hydrolysis or alcoholysis, such as with concentrated hydrochloric acid, methanol, and combinations thereof, gave similar results. It was found that only freshly drawn samples of the pentaborane-xylene solution could be depended upon to give results that came close to the Callery value of 543 micrograms of boron per ml of solution. Solutions drawn into glass stoppered graduates, originally kept under a nitrogen blanket and in a refrigerator, but opened a number of times in order to withdraw samples, soon showed white sediment which was proven by analysis to be boric acid, or something close to it (16.2% boron against 17.5% for boric acid). Also, stock solutions and standards prepared from the original Callery solution showed appreciable diminution of boron content, a phenomenon which is attributed to a certain water content of the xylene employed for making dilutions. The product of hydrolysis, boric acid, was noted as deposits on the walls of the volumetric flasks in many cases. This thought was further strengthened when dilutions were made with absolute methanol and xylene respectively and showed on analysis the calculated boron content for the methanol dilution and about 65% thereof for the xylene dilution. Additional work along these lines, with carefully dehydrated xylene, are strongly indicated and will be carried out as time becomes available.

A stock solution for standardization purposes was prepared from the original Callery solution so as to contain 217 micrograms of boron per ml, but was found on analysis to contain 192 micrograms. It was designated as a 200 microgram stock solution and was used for making further dilutions. The first attempts made with these dilutions to construct a standard curve were made by mixing 5 ml of the various pentaborane "standards" with 5 ml of 100% pyridine containing 0.1% of TTC. A 50:50 xylene-pyridine mixture was used as a blank and readings were made at the wavelength of 490 millimicrons, as indicated above. Results obtained plotted out on semi-log paper as a straight line, but the curve was in an undesirable instrumental range (90–100% transmittance). It seemed a logical conclusion that water is necessary to make the reaction go with reasonable speed. Orienting tests showed that 1 ml xylene mixed with 9 ml pyridine will yield a single phase on addition of up to 4 ml of water, a mixture of 1 ml xylene with 10 ml pyridine will tolerate up to 5 ml of water, and 2 ml xylene mixed with 9 ml pyridine will pick up a maximum of 3 ml of water before separating into two phases.

On the basis of these results it was decided to run a series of tests in which 1 ml each of several pentaborane-xylene "standards" would be mixed with 9 ml pyridine (100%) and 1 ml of 5% aqueous TTC. Pyridine was to be used as a blank in taking the readings. In these tests the pyridine was allowed to react with the pentaborane, as it will do, for five minutes before the TTC solution was added. Readings were taken 10, 30, 60 and 120 minutes after mixing. Results obtained for the 10-minute readings are shown in Figure 2 and the other data recorded in Table I. They show that straight line standardization curves can be obtained in a desirable instrumental range, and that the stock solutions degenerate with time, probably because of a cer-

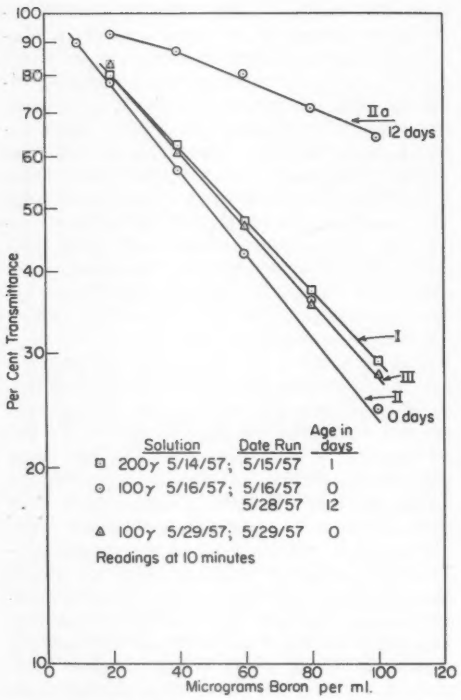

FIGURE 2. Influence of time on the pentaborane-TTC color reaction.

TABLE I

Pentaborane-TTC-Color Reaction
(% Transmittance with differing amounts of boron)

Curves	Stock solution, micrograms per ml	Date diluted	Date Analyzed	Micrograms of boron						
				0	10	20	40	60	80	100
I	200	5/14/57	5/15/57	96.3		80.0	62.4	47.3	37.5	29.2
II	100	5/16/57	5/16/57	94.2	90.0	78.0	57.2	42.7	36.0	24.7
IIa	100	5/16/57	5/28/57	96.8		92.4	87.2	80.5	71.2	64.5
III	100	5/29/57	5/29/57	98.2		83.0	60.4	47.2	35.6	27.8

tain water content of xylene, as mentioned before.

To further study the influence of water on the color development, a series of tests were made with pyridine-water mixtures of three different concentrations, viz., 70, 80, and 90% pyridine. A fresh stock solution containing 100 micrograms of boron was prepared from the original Callery pentaborane-xylene solution, and the stock was further diluted to levels of 20, 40, 60, 80 and 100 micrograms of boron per ml of xylene. In each case one ml of each dilution was was mixed with 10 ml of aqueous pyridine containing 0.01% TTC, in glass stoppered tubes, and the samples were mixed for one minute. The samples were read at 490 millimicrons after several time intervals, against an appropriate blank. Results obtained are given in Table II and are represented graphically in Figure 3. They show that the influence of water is very marked, up to a water content of about 20%, but that a further increase to 30% does not intensify the color sufficiently to be of significant advantage.

Utilizing the knowledge gained in this work, a fresh sample of pentaborane-xylene was drawn from the steel supply cylinder into a glass stoppered graduate cylinder flushed with nitrogen, and dilutions were made and TTC tests run in a manner described immediately above, except that 80% aqueous pyridine was used and that the spectrophotometer readings were made 60 minutes after mixing the reagent with the samples, using pyridine as a blank. Each dilution was run in duplicate and the figures were av-

TABLE II

Pentaborane-TTC-Color Reaction (influence of water content)

Micrograms boron	% Transmittance		
	30 mins	60 mins	120 mins
70% pyridine—30% water			
Blank	98.5*	97.9*	97.2*
20	78.4	77.8	77.8
40	55.2	53.9	53.5
60	40.6	39.2	39.2
80	27.5	26.0	26.1
100	19.5	18.4	18.5
80% pyridine—20% water			
Blank	98.1*	97.7*	96.8*
20	78.8	78.4	78.5
40	60.6	59.7	59.5
60	44.8	43.5	43.5
80	33.9	32.2	32.2
100	26.3	25.0	25.0
90% pyridine—10% water			
Blank	96.7*	96.3*	95.0*
20	93.0	92.1	92.2
40	84.7	83.3	83.1
60	75.0	73.2	73.1
80	69.4	68.2	67.3
100	61.7	59.7	58.9

* Blanks were read against pyridine; all other readings were made against the corresponding blank as 100%.

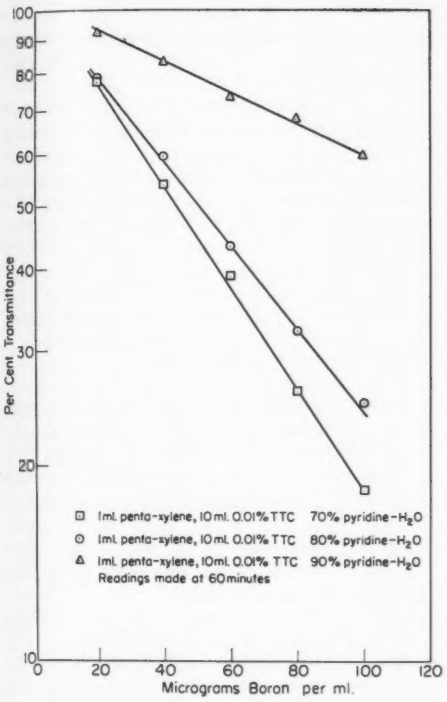
Per Cent Transmittance
Micrograms Boron per ml.
1ml. penta-xylene, 10 ml. 0.01% TTC 70% pyridine-H_2O
1ml. penta-xylene, 10 ml. 0.01% TTC 80% pyridine-H_2O
1ml. penta-xylene, 10ml. 0.01% TTC 90% pyridine-H_2O
Readings made at 60 minutes

FIGURE 3. Comparison of the influence of moisture in pyridine in the pentaborane-TTC color reaction using 70%, 80%, and 90% aqueous pyridine.

eraged for a "standard curve". Optical densities were recorded instead of transmittances in order to be able to correct for the absorbance of the blank by simple subtraction. The results obtained are presented in Figure 4. The curve is very close to others obtained in similar work not here reported, which shows that replication is satisfactory.

Upon addition of the pyridine directly to the pentaborane-xylene solution, a reaction occurs making the sample yellow. This color gradually increases and then decreases again after about 10–15 minutes. In order to see what effect this would have upon the color development with TTC, two series of tests were made in which the tubes containing pentaborane-xylene samples and pyridine were allowed to stand for from 0 to 60 minutes before addition of the aqueous TTC solutions (1 and 5% respectively). A pentaborane-xylene standard of 40 micrograms was used with the 5% TTC and a standard of 100 micrograms was used with the 1% TTC. Results obtained are given in Table III. They show that maximum color development is reached in from 5–10 minutes. To study the influence of the sequence of the several procedural steps for mixing the reacting components, three series of tests were made as follows. Appropriate amounts of stock solution of pentaborane-xylene (100 micrograms B per ml) and xylene were pipetted into tubes to yield one ml each of dilutions with 20, 40, 60, 80 micrograms per ml. In the first series of tests, one ml of aqueous 1% TTC was added to these dilutions, and then 9 ml of 100% pyridine. In the second series of tests, a mixture of one ml of aqueous 1% TTC and 9 ml of pyridine was added, and in the last series the 9 ml of pyridine were first added to the pentaborane dilutions and then one ml of aqueous 1% TTC. Results obtained are given in Table

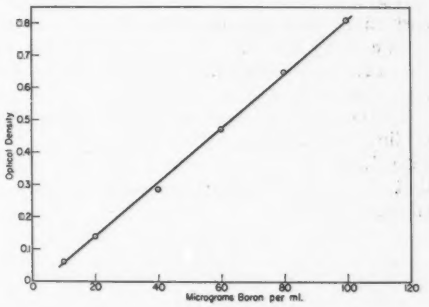

FIGURE 4. Standardization curve for the pentaborane-TTC color reaction.

TABLE III

Pentaborane-TTC-Color Reaction as Influenced by Prior Pentaborane Reaction with Pyridine

TTC added after	% Transmittance, minutes after mixing				
	0	30	60	120	1140
40 Micrograms B; 5% aqueous TTC					
0 minutes		69.7	63.2		
5 "		63.7	56.8		
10 "		55.3	50.0		
15 "		56.2	51.7		
25 "		62.1	58.8		
35 "		69.7	65.9		
100 micrograms B; 1% aqueous TTC					
0 minutes	69.0	44.0	31.5	28.2	30.2
5 "	43.0	26.5	21.8	19.6	21.4
10 "	43.0	26.5	23.0	20.6	21.9
20 "	55.0	39.2	35.0	32.2	33.5
40 "	78.2	66.0	66.9	—	58.2
60 "	93.2	92.5	91.6	—	78.3

TABLE IV

Pentaborane-TTC-Color Reaction as Influenced by Sequence of Adding Reactants

Micrograms boron	% Transmittance at 490 millimicrons											
	TTC added to penta, then pyridine				Mixture of TTC and pyridine added to penta				Pyridine added to penta, then TTC			
	10 min.	30 min.	60 min.	120 min.	10 min.	30 min.	60 min.	120 min.	10 min.	30 min.	60 min.	120 min.
Blank*	97.7	97.7	97.0	96.5	100.0	99.6	98.7	97.2	100.0	98.4	98.0	96.2
20	90.3	88.1	86.4	85.0	90.3	88.1	86.4	85.0	88.7	82.3	78.8	76.3
40	82.8	78.0	75.1	73.0	81.3	77.8	75.2	73.8	72.0	63.0	58.0	55.0
60	70.9	64.1	60.2	57.8	74.7	70.2	67.0	66.0	55.0	44.2	38.8	36.0
80	60.7	54.0	49.9	47.0	68.5	63.3	59.9	59.0	45.8	35.9	30.8	28.0
100	56.5	49.2	44.3	40.5	61.8	56.2	52.6	51.9	40.9	30.9	25.2	22.0

* Blanks were read against pyridine; all other readings were made against the corresponding blank as 100%.

IV. They show that in general greatest color depth is attained if the pentaborane solution is first mixed with pyridine, that the next best procedure is to add the aqueous TTC first, and that somewhat less color is produced if a mixture of aqueous TTC and pyridine is added.

The actual concentration of TTC seems to be of limited importance because extensive tests with 1, 3 and 5% aqueous TTC have shown that the color intensities are about the same at all concentrations.

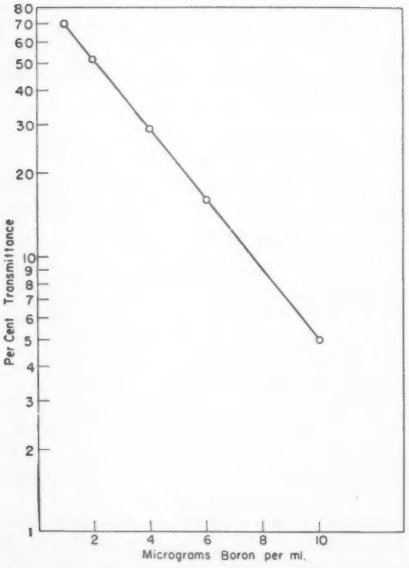

FIGURE 5. Standardization curve for decarborane-TTC color reaction in a two-phase system consisting of aqueous NaOH and xylene. The colored layer was centrifuged to clarify it.

Similar investigations were carried out to ascertain the usefulness of the TTC reaction for the determination of decaborane[4]. A stock solution containing 200 micrograms of B per ml was prepared by dissolving 0.0565 gram of decaborane of better than 99% purity in 250 ml of xylene. Dilutions were prepared from this stock solution in known manner, and they were made fresh each time they were needed.

The first effort was directed to the two-phase system, using aqueous alkali and TTC for the reaction with decaborane, and extracting the formazan with xylene. Mixing of the reacting components was carried out in glass stoppered tubes, adding to each 5 ml of the various concentrations of decaborane solution, 4 ml of water containing 1% TTC, 1 ml of 0.6*N* NaOH (normality not critical), and 15 ml of xylene. The solutions were mixed thoroughly for about 10 seconds, allowed to stratify, and the xylene layer was then transferred to standard cuvettes. An absorption curve established for the color thus produced proved that its maximum absorption was also at 490 millimicrons, just like that from pentaborane and TTC. Results obtained in this manner, when plotted on semi-log paper, gave a reasonably straight line, and it was surmised that what kinks it had were due to insufficient clarification of the xylene layer, emulsions having been observed in practically all tests.

This thought was borne out by the next series of tests in which the final mixture of reactants was allowed to stratify and the xylene layer was then centrifuged. Results obtained gave an excellent standardization curve, when minimum transmittance values were plotted. It is depicted in Figure 5. The principle was thus established that solutions obtained in the above manner follow Beer's law, and that a good analytical procedure for the determination of decaborane can be based on these findings.

The inherent tediousness of the two-phase method of operation, and especially the need for a centrifuging step, suggested that a single phase system would preferable for decaborane, too. Aqueous solutions of pyridine containing 10, 15, 20, 25 and 50% by volume of the latter were chosen as the reaction media, even though anhydrous pyridine proved suitable, because considerations of cost and of odor molestation seemed to make this course advisable. The lowest practicable pyridine concentration was found to be about 10%. At this level color development is very rapid, reaches a maximum and then diminishes again. The initially clear, red solutions gradually become cloudy, and a ring of red solid is eventually deposited on the cuvette wall at and near the liquid meniscus. The latter phenomenon would explain the fact that color development goes through a maximum. Apparently 10% aqueous pyridine is able to dissolve or peptize the formazan as it is formed. However, the particles seemingly increase in size gradually until flocculation occurs.

As a possible solution to the problem, attempts were made to aid the insufficient solvent action of 10% aqueous pyridine by the addition of small amounts of alcohols or dispersing agents. Isopropyl and tertiary butyl alcohol proved useful or the purpose, in amounts of 10% by volume of the reaction mixture, and straight line standardization curves were obtained with both of them. Of the dispersing agents used, Atlas G-1086 and Triton X-45, the latter proved the much superior one, and more extensive tests were made with it, and they are to be reported here in more detail.

A stock solution containing 200 micrograms of boron per ml was prepared by dissolving the appropriate amount of decaborane in 10% aqueous pyridine, and dilutions ranging from 5 to 40 micrograms per ml were made from it in customary manner. Similarly, a 1% TTC solution in 10% aqueous pyridine was prepared, and also a solution of 1% Triton X-45 in the same solvent. For each test, 5 ml each of the decaborane standards and of the TTC solution were mixed directly in the cuvettes, and one ml of the Triton X-45 solution was added. Spectrophotometer readings were made at 490 millimicrons. Results obtained are given in Figure 6, which shows that a straight line calibration curve is obtained with a slope which allows the determination of a reasonable range of decaborane concentrations. This work was repeated three times in order to see what replication of the results could be obtained, and in each case good agreement was noted. As can be seen from the curves, several hours should be allowed for full color

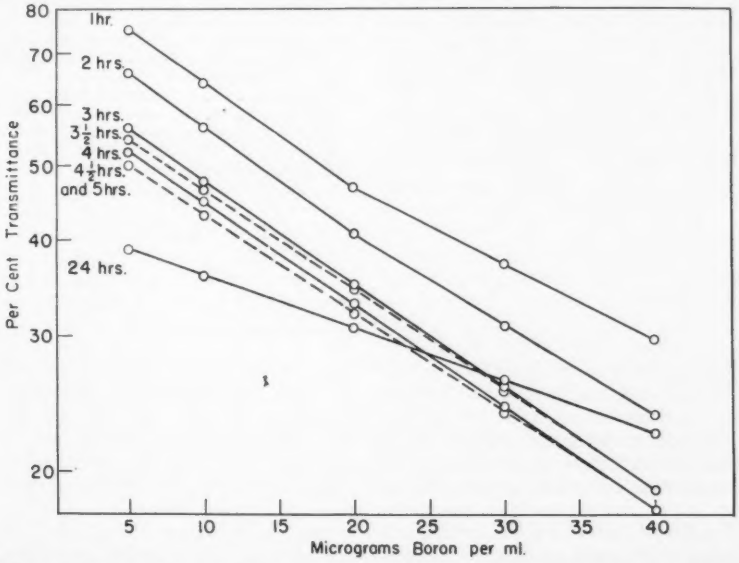

FIGURE 6. Standardization curve for decaborane-TTC color reaction using a one-phase system consisting of 10% aqueous pyridine with the addition of Triton X-45 to prevent precipitation of formazan. Color development is shown for several times up to twenty-four hours.

development of the higher decaborane concentrations, though at lower levels a waiting period of about one hour should suffice.

The concentration of the pyridine used as a solvent has a very pronounced effect. For substantially anhydrous pyridine the standardization curve is very steep, and on dilution with water the slopes become progressively smaller. This makes it possible to adjust conditions of operation with a view to either increasing the sensitivity or the range of concentrations of decaborane to be covered. The solvent power of pyridine for the formazan is naturally greatest when it is used in fairly anhydrous form, while with increasing water dilution the formazan is probably held only in colloidal solution and tends to separate out at levels of 15% pyridine or less, unless auxiliary solvents (*e.g.* isopropanol) or dispersing agents (*e.g.* Triton X-45) are used. It is entirely possible that the state of subdivision of the formazan, whether molecularly dissolved or colloidally dispersed, has a real bearing on the total amount of color developed, and the somewhat steeper curve obtained when either isopropanol or tertiary butanol was used in conjunction with 10% pyridine certainly points in this direction. More work along these lines would be very desirable but could not be undertaken at this time.

No great importance of the TTC concentration could be observed, just as was the case for the reaction between TTC and pentaborane. The concentration of 1% was chosen for most of this work because it supplied enough TTC for the full range of decaborane concentrations investigated, as shown by simple calculation. However, tests were made with TTC concentrations from 0.1% to 20%, with an indication that at concentrations of 10 and 20%, maximum color depth was not reached as readily as with concentrations of from 1 to 5%, though the total amount of color was greater. Any concentration from 1–5% should, therefore, afford maximum operating conditions.

The TTC reaction is applicable to diborane, pentaborane, and decaborane, and instruments for monitoring industrial atmospheres for these boranes have been devised by Kuhns[2] with the aid of this principle and are being marketed by the Mine Safety Appliances Company of Pittsburgh, Pa. It is probable that many derivatives of the boranes can likewise be determined in this manner, but this area has not been investigated particularly. In any event, the reaction is non-specific because the red formazan is formed by many reducing agents, and the method has been used by workers in the field of biochemistry for a variety of purposes. While a lot of work remains to be done to round out the picture, it was considered desirable to make available to industrial hygienists the data and experience accumulated over the years in working with this method.

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2. Kuhns, Larry J., R. H. Forsyth, and J. F. Masi: Boron Hydride Monitoring Devices Employing a Triphenyltetrazolium Chloride Reagent. *Anal. Chem. 28:* 1750 (1956).
3. Hill, William H.: U. S. Patent 2,848,307, Process for Detecting Presence of Boranes, issued August 19, 1958.
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Conference on Applications of X-ray Analysis

THE NINTH ANNUAL CONFERENCE on Applications of X-ray Analysis will be held August 10, 11, and 12, 1960 at the Park Lane Hotel, Denver, Colorado. Papers and discussion will be presented in the areas of diffraction, fluorescence analysis, absorption techniques, and instrumentation. A large number of papers have been submitted resulting in the selection of a full, well-rounded program. A variety of techniques will be involved, including microanalysis with soft x-rays and x-ray microscopy.

The Proceedings of this Conference will be published by Plenum Press, N.Y.C. Proceedings of the sixth, seventh, and eighth Conferences are still available from Plenum Press. For further information of this conference write to William M. Mueller, Metallurgy Division, Denver Research Institute, University of Denver, Denver 10, Colorado.

Multi-Operational Chamber for Calibration Purposes

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FREQUENTLY during industrial hygiene and air pollution work, a need arises for a chamber which can be used for calibration purposes. A multi-operational calibration chamber has been designed and built for this purpose. The chamber incorporates extreme flexibility in operation. Both static and dynamic operation are feasible within this chamber. Thus, both continuous and batch generation of gases, vapors, dusts and mists can be utilized to prepare any reasonable concentration of these materials within the chamber system. The mixtures that can be prepared are used to improve the accuracy of industrial hygiene and air pollution field studies, as well as permit research and development work in these fields. Specific uses for this calibration chamber include:

1. Calibration of air sampling and detection devices used in industrial hygiene and air pollution field studies.
2. Development of new air analysis instruments and sampling equipment.
3. Evaluation of air analysis techniques.

Construction Details

The multi-operational calibration chamber consists of three basic parts: the chamber, the fans, and the ductwork. The details of each are described below.

Chamber: The chamber, shown photographically in Figures 1 and 2, has a useful static volume of 33.4 cubic feet. The shape of the chamber was so chosen to obtain the greatest enclosed volume within the area which had been set aside for its installation. The rounded ends are purposely included to eliminate sharp corners or possible "dead spots" within the chamber. The chamber section itself consists of two hemicylinders connected by flat sections. It is fabricated to include a double wall construction with the inner wall made of 316 stainless steel (extra low carbon) and the outer wall made of cold rolled steel. Vermiculite is packed in the annular space to provide both thermal and sound insulation. Seven sampling and/or utility ports are provided around the center opening or viewing door. This door has doubly articulated hinges so that it pulls out parallel to the wall before swinging to the side. This action permits tight sealing without distortion of the door gasket. Five knife-type clasps are used to press the door shut. A 1 inch wide x ½ inch thick, closed-cell neoprene sponge rubber is used for the door gasket. Many gasket materials were tried in the shakedown trials of the chamber, but closed-cell neoprene was found to offer the best sealing properties for this construction.

A 2-inch drain valve is located at the lowest point of a slightly sloped chamber floor in order to facilitate washing of the chamber surfaces to eliminate adsorbed materials between runs. Strippable plastic films will be used if highly corrosive substances are studied. Even though all surfaces in the chamber and ductwork in contact with added materials are relatively inert to corrosive substances, the use of these films is considered justified.

There are two windows, each 8 x 8 inches, in the roof of the chamber, fitting flush to the inside surface, to serve as light-entry openings. If a set-up is made within the chamber, spotlights placed in these positions will illuminate the chamber to facilitate reading of enclosed instruments.

Fans: Two internal mixing fans and a centrifugal fan are provided. The internal mixing fans are opposed diametrically and give excellent mixing efficiency. They have 316 stainless steel blades and shafts. Each internal fan shaft is sealed against an .001-inch, oversized, graphite-impregnated bronze bushing in passing through the double wall construction to the fan motor. A 1725 rpm, 1/12 horsepower, open motor, is used to drive each internal fan. The motors are isolation-mounted for noise reduction on the outside of the chamber.

The centrifugal fan (down-blast discharge) type V, size No. 1, is run by a doubly-wound, ½ horsepower, two-speed motor, running at either 1725 or 850 rpm. The motor is belted to a variable speed drive to produce as much as a

Presented at the Twenty-first Annual Meeting of the American Industrial Hygiene Association, Rochester, New York, April 28, 1960.

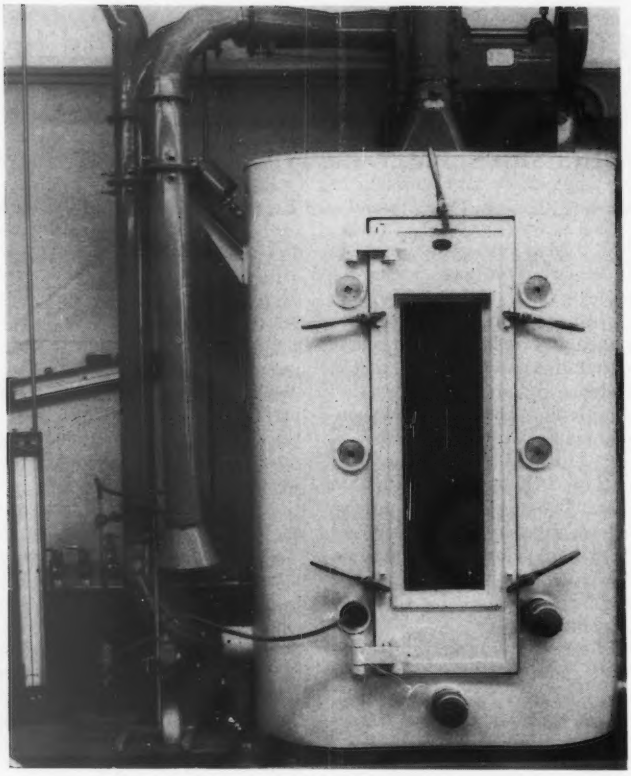

FIGURE 1.

6-to-1 ratio in fan speeds. The fan housing has a baked heresite coating on the inside for corrosion resistance. The fan wheel, LS type, is made of 347 stainless steel also for corrosion resistance. Minimum lengths of neoprene sheet are used in conjunction with steel strap to connect the fan to adjoining ductwork. The fan motor and drive are all isolation-mounted to eliminate vibration as much as possible.

Ductwork: The ductwork is fabricated to an inside diameter of 4½ inches from 16 gauge 316 stainless steel. Two precision-made orifices, meeting the American Society of Mechanical Engineers Specifications,[1, 2] are installed in the dynamic feed and the discharge lines of the ductwork. The orifices serve to meter the air when the dynamic system is being used. The second orifice serves as a permanent check on the accuracy of the other, and also as an indication of malfunction or leaks within the system.

Two butterfly valves, made of 316 stainless steel, are used to control air flow and to serve as shut-off devices when the static system is being used. In order to obtain a pneumatic seal whenever it is considered necessary, two butyl-rubber bladders are expanded in the ductwork on either side of the chamber proper. At six inches of mercury pressure in the bladder, complete closure was found to exist and this pressure has been established for normal operating procedure. In dynamic operation, these bladders are removed.

A diffuser is installed at the inlet to the chamber in the center of the roof of the chamber. It is shaped of perforated metal, as shown in Figure 3, to have a free area equal to the duct inlet area. Its design provides uniform air distribution to the chamber.

The discharge line from the chamber ductwork is directly connected to the building exhaust system. The building system exhausts above the roof level, thereby simplifying evacuation of the chamber contents.

Dynamic Operation

Dynamic operation is obtained by running the centrifugal fan with the two butterfly valves open. Air is sucked into the inlet duct (shown

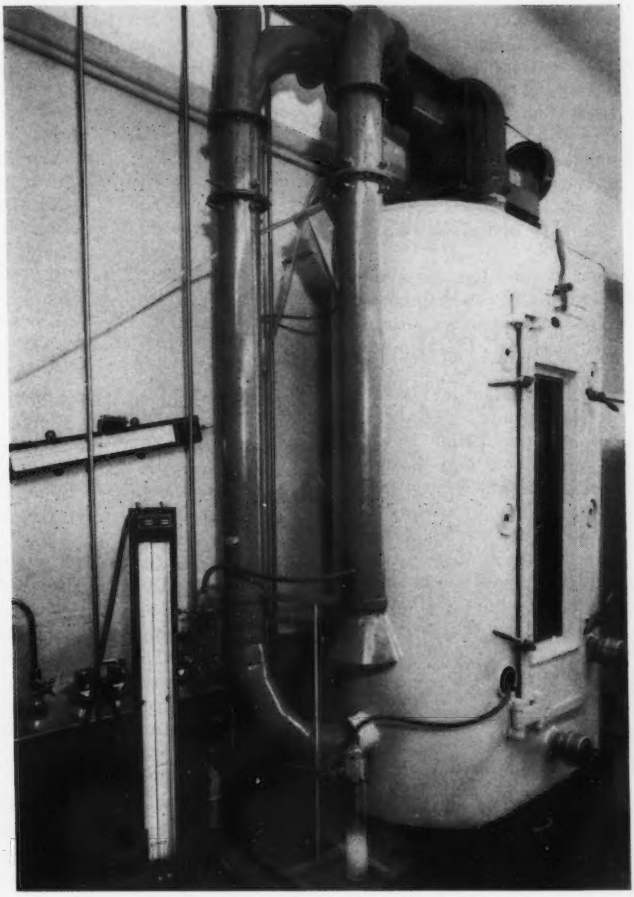

FIGURE 2.

with a 40° enclosed angle entrance hood) and passes through the centrifugal fan down through the diffuser into the chamber proper. The air then moves through the chamber, being mixed continuously with the internal mixing fans, and moves out through the outlet duct into the building exhaust system. Gases or vapors under study are simultaneously fed into the inlet duct at a known rate (see section on Feed Mechanisms). The combined gas or vapor air stream is metered by the orifices in the ductwork. The concentration developed is the ratio of the feed rates of the gas or vapor to the air. The air flow rate is obtained from the orifice reading in either the inlet or outlet ducts. Generally speaking, the small flow rate of the gas or vapor can be neglected in determining the air flow rate metered by the orifices.

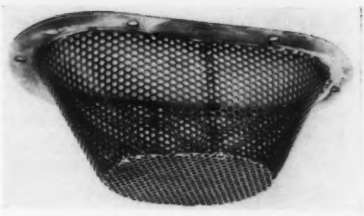

FIGURE 3.

Another method of dynamic operation which has been used successfully is shown in Figures 1 and 2. This system involves closing the outlet butterfly valve to the building exhaust system while leaving the inlet valve open. Gases or vapors are fed into the inlet duct as described

previously. The gas-air mixture is withdrawn at a known rate from a port, using an external source of suction like house vacuum. The inlet valve being open allows the necessary air supply for the mixture withdrawal. The ratio of the gas feed rate to the air withdrawal rate is then the concentration developed within the chamber. The figures show a typical set-up for determining collection efficiency of a gas in a Greenburg-Smith impinger. In this operation only the mixing fans are used.

In dynamic operation, consideration must be given to the fact that initially the chamber may not contain the desired concentration. Normally the chamber is filled with air. If one were to start sampling immediately, based on a feed concentration, erroneous results would of course occur. For the multioperational chamber described in this paper, at the maximum flow rate of about 120 cfm, it takes about two minutes to reach 99.9 per cent of the desired concentration. At lower air flow rates, the time to reach equilibrium is proportionately increased. For example at one cfm flow, it would take almost four hours to reach 99.9 per cent of the feed concentration.

When the experimental technique allows and time is important, a mathematical approach can be utilized to obtain the average concentration during the full operating period including the build-up time. In this way, long waiting times and wasted feed materials can be eliminated. A paper by Brief[3] describes in detail the mathematical procedures in problems of this sort.

With reference to this chamber, the formulae for calculating the average concentration when it is initially filled with air or when it has some concentration of the desired material are as follows:

C = average concentration
C_i = initial concentration, units same as C
C_s = supply concentration, units same as C
e = natural logarithmic base, 2.718 . . .
Q = total flow rate, cc/min.
R = chamber volume, cubic centimeters
t = time elapsed, minutes

For $C_i = 0$:

$$C = C_s - \frac{C_s R(1 - e^{-Qt/R})}{Qt} \qquad (1)$$

When Qt/R is large, the expression can be reduced to

$$C = C_s(1 - R/Qt) \qquad \ldots (2)$$

For $C_i \neq 0$:

$$C = C_s - \frac{(C_s - C_i)R(1 - e^{-Qt/R})}{Qt} \qquad (3)$$

TABLE I
Magnitude of Variables

Qt/R	$e^{-Qt/R}$	$(1 - R/Qt)$
9.2	.0001	.891
6.9	.001	.855
4.6	.01	.783
3.0	.05	.667
2.3	.10	.565
1.9	.15	.474
1.6	.20	.375

If Qt/R is large, the equation may be simplified to

$$C = C_s(1 - R/Qt) + C_i R/Qt \qquad \ldots (4)$$

Personal judgment based on the precision of measurements, accuracy desired, and the testing situation will determine whether use of equations (2) or (4) is justified. As a guide, the following table compares the magnitude of the various parts of the equations under consideration:

To illustrate the use of Table I, if one per cent accuracy (as listed in the second column) is required, then use of equation (2) or (4) is justified when $Qt/R > 4.6$. Within one per cent then, with $Qt/R = 4.6$ and the chamber initially free of gas or vapor, the average concentration will be 78.3 per cent of the supply concentration as can be seen in the last column.

Static Operation

Static operation involves feeding a known volume of material to the known volume of the chamber. The ratio of these volumes is then the concentration prepared. The internal mixing fans provide extremely rapid mixing so that the air-gas mixture is uniform throughout within a few seconds.

When sampling from a static system, the volume of sample withdrawn will either cause the pressure within the system to decrease or by air-infiltration cause a gradual decrease in the prepared concentration. It is possible to take advantage of this continuing dilution of the initial chamber concentration. Morley and Tebbens[4] developed a calibration scheme for air flow through electrostatic precipitators based on the air-infiltration dilution of a known concentration of CO_2 in a chamber.

To overcome "dilution" corrections, a collapsible bag could be inserted in the chamber and the exhaust from the sampler fed to the bag in a closed-loop system. In this way, the pressure within the chamber would remain constant and air infiltration would not be a problem.

Conversely, when developing very high concentrations within the chamber on a static basis, a recommended method is to inflate a plastic bag in the chamber so that it approaches the fraction of the chamber volume that is desired for the material. As the material under study is fed in, the bag is deflated until the desired concentration is achieved. Normally, high concentrations are developed dynamically, but if high concentrations are desired under static conditions, the inflatable bag technique may be used.

This chamber has been operated statically with 1.2 cubic feet of air removed (i.e., a vacuum of 15 inches of water) before infiltration occurred. That is, 1.2 cubic feet of sample of the initial concentration can be withdrawn during static operation. This undiluted sample volume far exceeds the volume necessary for many concentration calibration problems (e.g., indicator tubes and combustible indicators). For sample volumes exceeding about one cubic foot, dynamic operation is usually used.

Feed Mechanisms

Silverman[5] has reviewed the literature on the various feed mechanisms that may be used in preparation of gaseous mixtures for use within calibration chambers. Many of these have been tried and used with the multi-operational calibration chamber at Esso Research. Syringes and rotameters, however, have been found most reliable in preparing gas and vapor concentrations. A syringe driver for preparing gas or vapor concentrations under dynamic conditions has been used. Various size syringes can be driven at one of five different speeds, thus yielding a wide range of possible feed rates. If mercury does not interfere with the mixture under study, placing a layer of mercury in the syringe to cover the plunger is a very effective way of obtaining an absolute air-tight seal within the syringe.

None of the various feed mechanisms for dust and mist generation have been tried as yet for use within the chamber or its ductwork. Information developed in this regard will be presented in a future report.

Shakedown

The chamber was carefully checked for leaks prior to use with both a freon leak detector and soap solution. Freon 12 was added to the chamber and to the annulus. Pressurization with air then made it possible to use the freon detector to determine leaks. Leaks were further checked with soap solution after pressurizing both the chamber and the annular space.

TABLE II

Multi-operational Calibration Chamber Volume Determination by Three Methods

Method	Number of observations	Chamber volume, cubic centimeters
Mensuration	2	874,000
Gas concentration	2	1,060,000
Pressure change	9	946,000

Chamber Volume Determination

The volume of the chamber was determined by three independent methods: (1) linear measurement and application of geometric formulae; (2) feeding known volumes of propane into the chamber and measuring the propane concentration; and (3) changing the pressure in the chamber and measuring the volume of air that created the pressure change. The results from the three methods are shown in Table II.

The first method involved linear measurement and application of geometric formulae. This method required some estimation because of some odd shapes such as the duct intersections, the adapter between the fan and the chamber, and the sloping floor within the chamber. The volume of the fan housing was obtained from the manufacturer.

The second method involved feeding a known volume of propane into the chamber and then by mass spectrometric means determining the propane concentration of samples of the chamber air. The chamber volume was then calculated by simply dividing the concentration found into the volume of propane delivered. The apparently high value for the system volume obtained by this method could be attributed to gas adsorption on the chamber walls. The resultant lower concentrations then yield a higher calculated chamber volume.

The third method was the most unique and was considered to be the best of the three methods used. It involved changing the pressure in the chamber and then while returning the chamber to its initial pressure, collecting the inspired or expired air in a calibrated water-sealed gas holder or spirometer. The volume collected was measured and the chamber volume was then calculated directly from equation (5) below which is derived from the perfect gas law and the equation of continuity.

$$V = \frac{P\Delta V}{\Delta P} \qquad (5)$$

where:

V = chamber volume
ΔV = air volume at pressure P causing chamber pressure change ΔP, same units as V
P = absolute initial pressure in the chamber (practically set at atmospheric pressure)
ΔP = change in chamber pressure equivalent to ΔV, same units as P.

In transferring the air from chamber to spirometer or visa versa, it was noted that the high velocity of air flow through the small interconnecting tubing used created a cooling effect. This temperature change then made it necessary to either measure temperature and apply correction factors or allow the system to return to its initial equilibrium. This thermal problem was easily overcome by connecting the chamber and spirometer with a relatively large 3-inch line. The pressure in the chamber was varied from 382.5 to 437.6 inches of water with a barometric pressure equaling 407.6 inches of water. Results for nine different pressures within this range all gave results that clustered around 946,000 cubic centimeters. The 95 per cent confidence interval for the results was 938,000–954,000 cubic centimeters.

It should be noted that equation (5) might have application in volume determination of any shape not easily amenable to mensuration but able to withstand some pressure change. Any total volume meter like a wet test meter or a gas holder could be used to measure the change in volume, ΔV, corresponding to the pressure change developed, ΔP. Knowledge of the initial or barometric pressure then allows use of equation (5) to solve for the space volume directly.

Summary

The chamber system herein described is finding extensive use in calibration and development work in the fields of industrial hygiene and air pollution. The fact that the chamber can be operated either statically with an approximate million cubic centimeter capacity or dynamically with a wide range of air flows makes for its useful versatility.

Acknowledgment

The helpful suggestions of Dr. Leslie Silverman of the Harvard School of Public Health are hereby acknowledged with thanks.

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International Congress on Occupational Health

THE THIRTEENTH INTERNATIONAL CONGRESS on Occupational Health will meet July 25 through 29, 1960 at the Waldorf-Astoria Hotel in New York City. This Congress has been mentioned in several previous issues but now it is only a few weeks off. If you have not made your arrangements to attend, you had better move fast. Write to Dr. R. E. Eckardt, P.O. Box 45, Linden, New Jersey, for information and advance registration.

Persons of many nationalities and languages will be in attendance and on the program. Abstracts of papers will be distributed in English, French, German and Spanish at the meeting, and the entire proceedings will be published later. Simultaneous translation in the four languages will be provided at the plenary sessions but not at the concurrent technical sessions. Plenary sessions will be held on the first morning and the final afternoon of the Congress. One afternoon (Wednesday) will be devoted to plant visits and other entertainment. The other mornings and afternoons each will be devoted to five concurrent technical sessions covering all phases of occupational health. A fine program of hospitality is planned for accompanying spouses.

Among the many topics to be covered by multiple papers in the technical meetings are heat and strain, mental health, lead poisoning, pneumoconioses, noise, vibration, illumination, toxicology, analyses, physiology, dermatology, ionizing radiation, legal aspects, plant health services, maximum allowable concentrations, and a host of others. This is a notable chance to hear, meet, and talk with outstanding industrial hygienists from the world over.

The Control of Beryllium Hazards

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Introduction

WITH the exception of certain radioisotopes, the toxicity of beryllium probably exceeds that of any material previously used in industry. Consequently, it has been necessary to develop special health and safety control procedures for its safe handling. This Laboratory has devoted considerable effort to the application of enclosure techniques developed for radioisotope control to the control of beryllium.

This report has been prepared in answer to requests for information regarding our procedures. It is hoped that the information will be of assistance to those who are investigating beryllium safety measures preparatory to undertaking work involving beryllium and its compounds.

Toxicology and Permissible Concentrations

Although beryllium and compounds of beryllium in dust form may cause dermatitis[1] or beryllium granuloma and skin ulcers[2] following skin cuts and abrasions contaminated with beryllium, the principal hazard of beryllium is due to the toxic properties of inhaled dusts.[3]

Two types of respiratory damage, acute pneumonitis[1] and chronic pulmonary granulomatosis,[4] have been seen in the beryllium industry occurring among workers exposed to beryllium dusts, fumes, and mists.

The acute beryllium pneumonitis is similar in many ways to a chemical pneumonitis such as might be caused by the inhalation of corrosive chemicals. In both cases the effect is inflammation of the upper respiratory tract and, in extreme cases, involvement of the entire pulmonary system.

Acute beryllium pneumonitis may follow single short-term exposure to relatively large quantities of beryllium with usually prompt symptoms of damage. While there are reports of fatalities resulting from acute exposures, recovery when made is usually complete with no apparent permanent ill effects. Infrequently, the acute form of the disease progresses into the chronic.

Chronic pulmonary granulomatosis is usually a delayed disease and is more subtle in its disclosure, some cases manifesting themselves several years after exposure. The chronic pulmonary form is usually characterized by the presence of a diffuse pulmonary reaction with scattered granulomatous lesions throughout the lung. The symptoms of chronic berylliosis do not differ greatly from other types of pneumoconiosis. The first symptoms may be a mild and vague indisposition, slight but persistent loss of weight, loss of appetite, labored breathing accompanying even modest exertion, and general lack of energy. As the disease progresses there usually is a gradual decrease in vital capacity. The disease is often fatal. At present considerable progress is being made with chemotherapy agents to arrest the pulmonary deterioration, but as yet no specific cure has been found.

As noted above, chronic berylliosis exhibits marked variation in the time required for manifestation. The usual time interval appears to be from three months to six years. Delay times up to 16 years have been noted.[5] Likewise, people differ greatly in respect to sensitivity toward beryllium dusts. Some have worked in atmospheres containing 30 milligrams of beryllium per cubic meter without appearance of acute cases, whereas in other plants 4 milligrams per cubic meter produced a high incidence of acute berylliosis with several fatalities.[6]

As a result of these fatalities attending the case histories of individuals exposed to beryllium dusts, the Atomic Energy Commission formed a Beryllium Medical Advisory Committee in 1948 to review the problem and to establish maximum permissible concentrations of beryllium in the air as guides for their contractors. These MPC values, which were published by the AEC on August 10, 1951, were as follows:

1. The in-plant atmospheric concentration of beryllium should not exceed two micrograms per cubic meter as an average concentration throughout an 8-hour day.

2. Even though the daily average might be within the limits of Recommendation 1, no personnel should be exposed to a concentration greater than 25 micrograms per cubic meter for any period of time, however short.

This work was done under the auspices of the U. S. Atomic Energy Commission.

3. In the neighborhood of a plant handling beryllium compounds, the average monthly concentration should not exceed 0.01 microgram per cubic meter.

Although these values have been annually reviewed there have been essentially no changes since the recommendations were originally published. They appear to be reasonable from a compliance standpoint and adequate protection-wise in that the number of reported cases of berylliosis has been markedly reduced in spite of a large increase in the amount of beryllium and beryllium compounds processed.

The U. S. Atomic Energy Commission[7] has recently established the Hygienic Guide Series[8] as the responsible reference for maximum permissible concentrations of airborne beryllium. The wording of this reference is as follows:

Occupational 8-hour:	0.002 milligram beryllium per cubic meter of air.
Occupational, acute (less than 30 minutes):	0.025 milligram beryllium per cubic meter of air.
Non-occupational 24-hour:	0.00001 milligram of beryllium per cubic meter of air.

Enclosure Technique for Containment of Beryllium Dusts

Aside from the obvious dust potential from handling beryllium metal or compounds in powdered form, the most likely source of atmospheric contamination arises when such material is machined. Any such operation generates to a varying degree particles which are small enough to be airborne and be moved by gentle air currents. These particles can remain airborne near the operation long enough to be inhaled by the operator or by others nearby. The only effective method for preventing the spread of such contamination is to contain and remove the dust at its source.[9, 10]

In the enclosure technique the worker and the environment are protected by maintaining the zone of dust generation at a negative pressure with respect to the area occupied by the worker. This is accomplished by exhaust ventilation augmented by total or partial secondary enclosure. By total enclosure is meant one which completely encloses the zone of dust generation permitting no direct working opening to the outside. All necessary hand work is done through gloves mounted on the enclosure frame. Circumstances may require enclosing the entire machine or operation if the geometry, necessary machine movements, or the manual manipulations do not lend themselves to an enclosure fitting only the dust-generation zone.

By contrast, partial enclosures may surround the zone of dust generation or the entire operation but do give direct open access to the work by means of front-opening canopies, sliding doors, or plastic curtains. Partial enclosures are usually constructed on a frame with solid top, bottom, back, and sides, and normal work access is made through the front via movable sections. Depending on the operation, sliding doors may exist in the sides or the top of the enclosure for passing work in or out but are normally closed during operation. To illustrate by examples: a chemistry hood is a partial enclosure while a gloved box (dry box) is a total enclosure.

All enclosures are ventilated. Inlet air for total enclosures is filtered to reduce the dust load on the high-efficiency exhaust filters, and to prevent contamination of environmental air by out-breathing if the enclosure becomes pressurized because of accident or loss of suction. Partial enclosures require larger air flows than total enclosures. Essentially, controlled directional air flows, achieved by the maintenance of regional pressure differentials, are utilized to restrict dust to the enclosure. Consequently, in partial enclosures local exhaust ventilation is added whenever feasible within the enclosure to assure the proper pressure gradient. Ideally, the exhaust is close-capture in design, meaning that the dust pickup throat is in close proximity to the work and that guidance of the dust into the pickup is controlled by high face velocity.

Close-capture pickups in conjunction with "knockout pots" or dust-particle accumulators preceding the main filters permit recovery of material if this is required. In addition, this technique minimizes general contamination of the enclosure and is an important principle of "concentrate and confine" philosophy. Close-capture pickups are widely used to collect corrosive fumes if acids or other chemicals are present within the enclosure. In this case, the close-capture system exhausts through a scrubber to protect the filter and external duct work.

Local exhaust ventilation in the form of close capture is mandatory if dusts are generated without enclosure protection, is preferred with partial enclosures, and is optional with total enclosures with the exception of the special cases of recovery intent or corrosive fume production as noted above.

A minimum face velocity must be maintained across the open area of a partial enclosure to assure dust containment. This velocity will vary depending on enclosure design and the operations performed. Test smoke generated within the en-

closure furnishes a simple method for establishing face velocity requirements.

The general purpose of the enclosure philosophy is to isolate the contamination from the worker rather than isolating the worker from the contamination, and, since this is achieved by negative ventilation gradients, considerable engineering care should be taken in the design of the building ventilation. As previously noted, the close-capture zone should be at a negative pressure relative to the secondary container, and the secondary enclosure should be maintained at a negative pressure with respect to the area occupied by the worker. Furthermore, the shop or laboratory in which beryllium work is being performed should be at a negative pressure in respect to adjacent shops or laboratories. This is achieved most easily by introducing building make-up air in hallways or bays and thus assuring that these areas are pressurized with respect to laboratories and shops. This procedure has markedly minimized the potential building-wide contamination if local containment fails as might be occasioned by a fire or an explosion.

Dust collection by high-efficiency[11] filtration is of prime importance in the enclosure approach. These filters, placed in close proximity to each dust-generating operation, protect the manifolding from being grossly contaminated and, of course, are of paramount importance in removing hazardous dust from effluent air exhausts. Where possible, in particularly hazardous operations, each segment of the operation should be provided with its own exhaust manifold so as not to contaminate the entire shop manifold if a filter ruptures.

The filters are changed when the buildup of dust prevents the flow of sufficient air to properly ventilate the dust-producing zone. When this occurs, the dust-loaded filters are removed, bagged, and disposed as contaminated waste.

As an aid in determining the need for changing filters, pitot tubes are installed within the exhaust manifold to give continuous indication of flow through the filter. When a new filter is installed, a manifold damper is adjusted to give the desired face velocity at the enclosure opening. The flow reading of the rotameter connected to the pitot tube is recorded. As dust accumulates on the filter, the damper is opened to maintain the original flow. When it is impossible to maintain the proper flow with the damper fully opened, the filter is replaced.

Choice of enclosure type depends on the potential hazard of the operations to be performed, the movements of the operator, and the motion of the machine. Certainly visual or measurement inspection of freshly machined solid forms, if free of loose surface contamination, do not require total enclosure. This operation may be done in the open or at most requires only an open-front inspection box. Lathe work on solid elementa beryllium may usually be placed in a partial en closure, similar to that shown in Figure 1, if ma

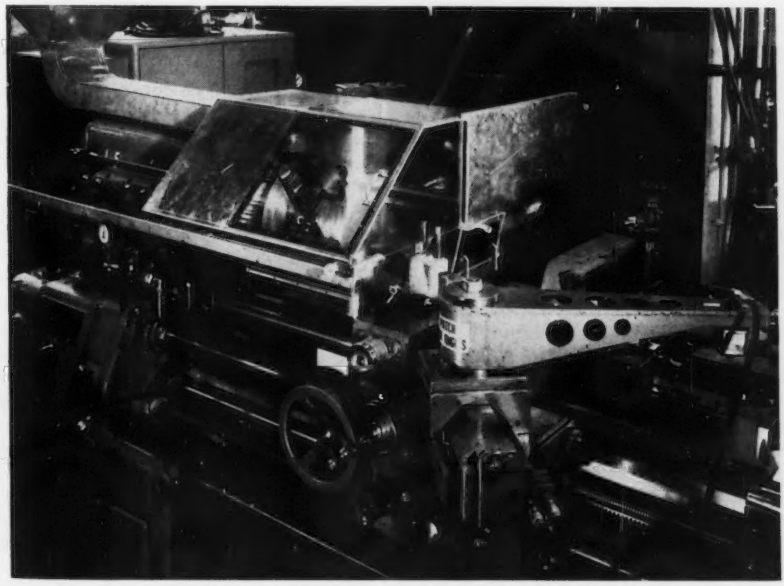

FIGURE 1. Lathe fitted with a partial enclosure.

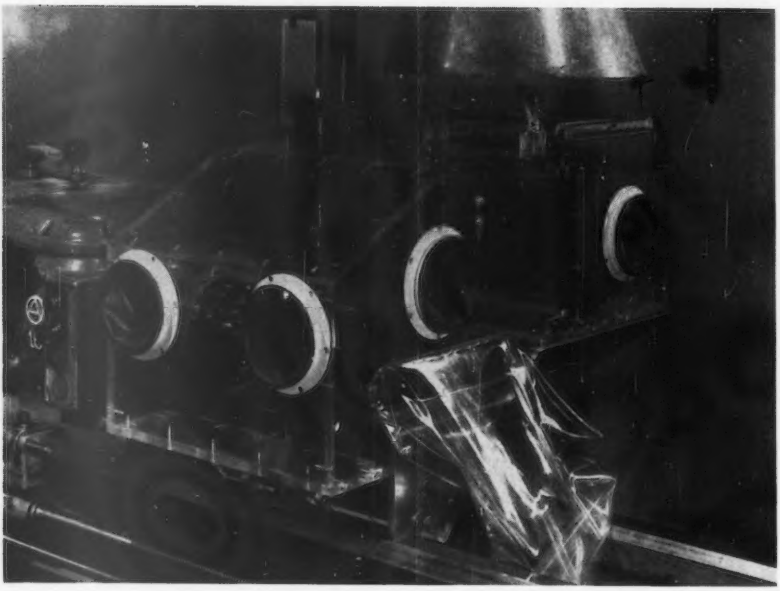

FIGURE 2. Totally enclosed lathe.

chining is performed with the aid of a coolant. If dry machining is employed the lathe should be totally enclosed. Such a lathe enclosure is shown in Figure 2. Beryllium welding should be done in a total enclosure; a prefilter (several types are available) should be provided to remove smoke or fumes and thus prevent rapid plugging of the high-efficiency filter. Milling operations, due to the degrees of machine motion, generally must be placed in a partial enclosure. Grinding operations are preferably totally enclosed. Processing of beryllium oxide in the powder form should always be totally enclosed.

Figure 3 shows a pictorial schematic drawing illustrating a typical gloved box enclosure system. This enclosure is suitable for processing powdered beryllium oxide. The bag-out port (Item 13) is a useful means for removing surface contaminated material from the enclosure. The port is fitted with a retaining groove for a plastic bag held in place by an "O" ring. Material is placed within the bag, the plastic is thermally sealed between the material and the port, the plastic is cut along the thermal seal, and the bag is removed for transfer into another enclosure or disposed of as waste. The terminal plastic on the port is covered by a new plastic bag which is secured to the port using the same technique as is used for changing gloves. The terminal plastic is then placed within the enclosure and disposed of via a waste drum attached to the enclosure or by subsequent bagging-out transfers. Although this appears involved, the manipulations become routine and the procedure is quite effective in eliminating environmental beryllium-oxide-powder contamination.

Considerably more care is required for partial enclosures where the area of the opening is adjustable than is required for total enclosures. Many cases have been noted in which doors, panels, etc., were left open when potentially hazardous operations were resumed after adjustments made by the operator in the course of the operation. The resulting deficiency in face velocity endangers the operator and his co-workers, because the reduced velocity allows the contamination to escape the enclosure.

Other Protective Measures

With well-designed, properly operating equipment, contamination is restricted to enclosures. The floors, machine surfaces, and the general working areas are normally uncontaminated and any loss of containment is localized and should be cleaned promptly. Respirators are worn only in special cases of work not conforming to enclosure or in cases of accidental failure of enclosures. Protective clothing is used but does not constitute a first line of defence against contamination.

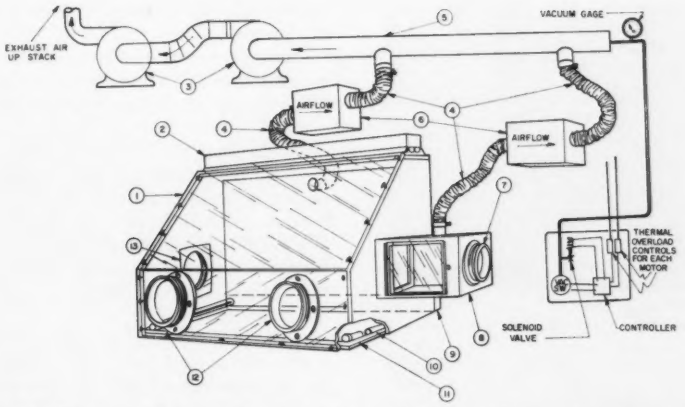

FIGURE 3. Pictorial schematic drawing of a typical gloved-box enclosure system. Numerals identify features as follows:

1—Airtight enclosure with Lucite view panel (constructed of plywood, metal, or fiberglas)
2—Exterior light
3—10 P ILG ⅓ hp centrifugal fan
4—Two-inch diameter flexible ducting
5—Four-inch exhaust manifold (if used)
6—GL filter, absolute plus PF 345 and PF 105
7—Five-inch gloveport ring to fit five-inch neoprene gloved-box type gauntlet gloves
8—Ventilated air lock separated from main enclosure by a sliding door
9—Fiberglas PF 105 inlet filter
10—Air inlet diffusion tube
11—Metal catch pan
12—Eight-inch gloveport ring to fit eight-inch neoprene gloved-box type gauntlet gloves
13—Bag-out port.

In areas where beryllium is processed it is recommended that floors be surfaced with smooth material such as linoleum to facilitate cleaning and that a routine janitorial program be maintained. Floors should be heavily waxed and should be damp-mopped between wax applications.

Beryllium Monitoring

Air Monitoring

A continuing air monitoring program is required to evaluate the performance of enclosures and to assure that hazardous concentrations of beryllium are not released to the room atmosphere. With adequate enclosures, no difficulty has been encountered in maintaining the average beryllium concentration below 0.2 $\mu g/m^3$ in working areas. As a consequence any incipient loss of containment is easily detected and signifies enclosure difficulties. As a working rule, a beryllium concentration of about 0.2 $\mu g/m^3$ is considered as an "alert" and immediate attempts are made to remedy the situation.

At this Laboratory environmental samplers for measuring occupational routine concentrations are operated on a 24-hour, 7-day week basis, drawing air through 10.3-centimeter-diameter paper at a flow rate of 4 cfm. Papers are changed daily Monday through Friday. The filter paper is identified as Staplex TFA-41 and the air mover is a modified Gast Model 0740 air pump. This sampler unit is shown in Figure 4. Spot, short-duration samples are taken at breathing-zone heights at potentially hazardous locations or during new unevaluated operations. These samples are taken with a Staplex Hi-Vol Sampler at flow rates of 10 to 20 cfm.

A program is currently under way to determine airborne beryllium on a continuous basis in order to improve the detection of momentary high concentrations of beryllium. Short-term samples, although superior in this respect to a sample collected continuously over an 8- or 24-hour period, still do not reflect the time-dependent factor as recommended by the occupational acute MPC. No sampling program has yet successfully shown

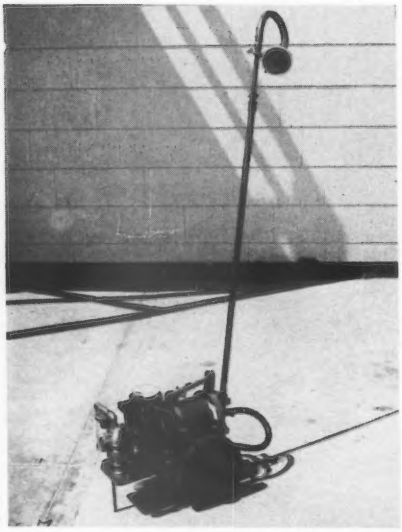

FIGURE 4. Environmental sampler for routine measuring of occupational exposure concentrations.

the exact time during the sampling period that the beryllium was collected on the paper. Consequently, one cannot be sure in a 100-cubic-meter sample containing 30 micrograms of beryllium that the beryllium was not all in the first cubic meter collected. If so, the occupational acute value would have been exceeded while the occupational routine value as normally calculated from the data would have been 0.30 $\mu g/m^3$.

In practice, while continuous indication of airborne beryllium concentration would be of assistance, lack of such information is not too serious because operations with contamination potentials are usually well recognized in advance. Consequently, workers know when to expect trouble and can take appropriate measures. Furthermore, a Hazards Control Representative, who is usually in attendance, can assist in evaluating the safety problems inherent in a new operation. The fact remains, however, that no continuously indicating beryllium air monitor is employed and development effort is under way to satisfy this need. The Harwell continuous spectrographic monitor[12] would appear to meet the requirements ideally. Unfortunately, we have not been successful in duplicating the reported performance of this unit; private communications with other interested groups indicate that our experience is not unique.

Despite the difficulties encountered, the Harwell approach offers sufficient promise to warrant further work. In the meantime, however, several other rapid spectrographic methods are being investigated.

One of these uses a paper which has been impregnated with a standard prior to air sampling. The grating monochromator has two exit slits; one set for the internal standard line, and the other for the beryllium line. The sample paper containing the standard plus beryllium contamination is sparked for 15 seconds, during which time the signals from both lines are integrated. The beryllium concentration is determined by the beryllium to standard signal ratio. Equipment for the method has been assembled and is currently under evaluation.

Another method for determining the beryllium content on a filter paper is through detection of the 4.5 Mev gamma emitted from the excited C^{12} nucleus following a Be (α, n) reaction. This method,[13] as developed at the Armour Research Foundation, uses several curies of polonium as an alpha source with the gamma radiation detected by a sodium iodide crystal scintillator. Work is in progress at this laboratory to duplicate the previous results and to attempt to improve the sensitivity and accuracy of the method.

Samples also should be taken downstream from prevailing wind either outside buildings processing beryllium or at perimeter property stations to determine that concentrations exceeding the non-occupational 24-hour MPC are not reached.

Surface Contamination

Floors and working surfaces should be "swiped" regularly to determine if contamination has occurred. Whenever swipe samples show detectable amounts of beryllium, the area is considered contaminated and decontamination is undertaken.

Analysis of Samples for Beryllium

At present the determination of beryllium in all samples is based upon a fluorometric method using the fluorescence produced by beryllium with morin dye.[14]

Summary

The enclosures as described have been applied to a wide variety of operations ranging from chemical processing of finely divided beryllium oxide powder to machining of elemental beryllium castings. No operations thus far have been accompanied by average daily air concentrations exceeding 2 μg of beryllium per cubic meter.

The importance of matching the degree of enclosure with the degree of hazard cannot be overemphasized. However, it is mandatory that continual review and surveillance be maintained to

assure that enclosures designed for low-hazard operations are limited to those operations. If programmatic changes that involve more-hazardous operations occur, then the enclosure must be modified accordingly.

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Western Industrial Health Conference

THE ANNUAL WESTERN INDUSTRIAL HEALTH CONFERENCE will be held October 7 through 9, 1960, at the Jack Tar Hotel, San Francisco, California. This conference is an annual gathering of Western Industrial Medical Association, Western Industrial Nurses Association, American Industrial Hygiene Association, American Society of Safety Engineers, and American Conference of Governmental Industrial Hygienists. The program includes papers of original work, many of which are not presented at other meetings, and a variety of round-table or panel discussions of all phases of industrial hygiene. The meeting affords an excellent opportunity for industrial hygienists and others in the western states to become acquainted and to share their problems and solutions. Because of distance, many of these people can attend national meetings but rarely. For further information write to Dr. D. H. Robinson, 225 Bush Street, San Francisco, California.

Industrial Hygiene Foundation

THE INDUSTRIAL HYGIENE FOUNDATION will hold the Twenty-fifth Annual Meeting at Mellon Institute, Pittsburgh, Pennsylvania on October 26 and 27, 1960. The morning of the first day will be devoted to papers directed to management. A joint session including medical, engineering, chemical-toxicologic, and legal representatives will be held that afternoon. On the second day these four groups will meet separately and concurrently to hear individual papers and round-table discussions in their special areas of interest. These Annual Meetings of the Foundation have been established for many years as one of the important gatherings of industrial hygienists. Those unfamiliar with these meetings may secure further information from the Industrial Hygiene Foundation, Mellon Institute, 4400 Fifth Avenue, Pittsburgh 13, Pennsylvania.

Lead Fume from Welding on Galvanized and Zinc-silicate Coated Steels

W. LEAK PEGUES

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THE high incidence of plumbism associated with the removal of paint containing lead from steel by use of oxy-acetylene torches is well known.[1, 7] However, little data are available regarding the potential fume hazard produced by welding and cutting of steel previously coated with one of the modern zinc-silicate applications or hot-dipped galvanized. These coatings have been very effective corrosion preventatives and are being used with increasing frequency in industry, especially in the coastal areas. Welding on previously coated steel is increasing in proportion to the general use of these coatings. An investigation was made to determine whether or not welding and cutting operations on steel with these coatings could give rise to hazardous amounts of lead and zinc fumes.

Lead and zinc occur in the coating compounds in various amounts. Slab zinc (spelter) used in the hot-dipped galvanizing process investigated was prime western zinc which has an ASTM specification of 98.30 per cent by weight of zinc, 1.60 per cent by weight of lead (maximum), and 0.08 per cent by weight of iron.[2] It was assumed that no additional lead was added in the galvanizing process.

The zinc-silicate coating investigated did not list lead as a component in the formulation, and it was necessary to analyze the coating used in this investigation. The preparation investigated was supplied in two containers. One contained a so-called reactive liquid and the other a dry powder to be mixed at the time of application in the following way: one gallon of powder to be mixed with approximately two thirds gallon of reactive liquid. This mixture was applied to cover 300 square feet of cleaned steel surface. The powder portion was analyzed and found to contain 12 per cent by weight of lead or an equivalent of 29 milligrams of lead per square inch of coating.

Since lead fumes have a greater toxic potential than zinc, greater emphasis was given lead in the investigation. The accepted maximum allowable concentration (MAC) values for lead in work room air is 0.20 milligrams per cubic meter, and for zinc oxide the MAC is 15 milligrams per cubic meter.[3] It is known that at about 500°C the vapor pressure of lead is high enough to produce a lead fume concentration equivalent to the MAC value.[1] During welding and cutting operations, temperatures may reach 1000° to 3000°C. At these temperatures the lead forms lead oxide on the surface. The oxide is vaporized even more readily than the lead. This situation may result in lead fume and dust concentration far in excess of the MAC value. The zinc in the coatings also volatilizes into zinc fume at welding temperatures. While the MAC value for zinc is relatively high, this value is easily exceeded by welding and cutting because of the high content of zinc in these coatings.

Sample Location

The investigative work was accomplished in two large buildings designated as the pipe welding shop and the structural shop, which were equipped with large doors, high ceilings, windows at two levels and overhead fans to supplement natural ventilation. Ventilation in the shops was excellent, probably much better in this respect than in most welding shops. Samples designed to show the effect of good ventilation were collected during the summer when ventilation was best. Other samples were collected in an enclosure to demonstrate the maximum fume concentration as in a confined area. A tarpaulin six feet wide was used to form an enclosure six by ten feet, open at the top. Twenty-one air samples were collected in this confined area.

Sampling Procedure

Samples were collected on Whatman No. 42 filter papers held by a funnel shaped test paper holder.[4] No. 42 paper was selected because it is known to have a fume collection efficiency of 96.8 per cent at a sample rate of 10 linear feet per minute.[5] Since compressed air was conveniently available an air ejector was utilized as a source of vacuum. A Fischer and Porter "Triflat" rotameter was placed between the filter paper and the air ejector to measure the sam-

ple volume. In tests where it was desirable to sample the fumes at the welder's breathing zone, a ¼-inch I.D. polyethylene tube three feet long was attached to the filter holder and used as a probe which was inserted into the welder's hood.

Analytical Procedure

The quantities of the metal oxides (iron, zinc and lead) retained on the filter papers during the sampling step were determined by standard procedures. The oxides were dissolved and washed from the filter paper with 1% nitric acid. The polyethylene tube used as a sample probe was rinsed with 1% HNO_3 and the rinsings added to the sample. An aliquot of the wash was then evaporated to leave total metal oxides. Iron was determined colorimetrically with ammonium thiocyanate solution by comparing the color developed in an aliquot to a standard having a known iron content.[6] Lead was determined colorimetrically by an adaptation of the method recommended by the American Conference of Governmental Industrial Hygienists. In this procedure lead is separated from an aqueous solution by the formation of the divalent lead salt of dithizone (diphenylthiocarbazone) at a pH of 9.3. After extracting with chloroform, the metal in the chloroform phase is determined by spectrophotometric measurement of the color-complex at 510 millimicrons. Interfering substances such as copper, iron, tin and zinc are eliminated by addition of hydroxylamine hydrochloride. It was assumed that the only other metal oxide present in significant amounts was zinc and this was determined by subtracting iron oxide and lead oxide from the total oxide present.

Results

The results of this investigation are presented in four tables showing analyses of samples taken

TABLE I

Experimental Confined Area (Poor Ventilation)

Coating	Type weld	Location of sampling probe†	Fume concentrations, mg/M³					
			ZnO	Avg.	Fe_2O_3	Avg.	Pb	Avg.
Zinc-silicate	Elect. arc	2′ directly above welding.	159.9		38.9		15.2	
" "	" "	3′ above and 2½ back of welding.*	107.2		11.9		0.86	
" "	" "	3′ above and 2′ back of welding.*	68.9	119.9	11.8	24.2	3.27	5.63
" "	" "	3′ above and 2′ back of welding.*	64.0		12.6		3.65	
" "	" "	Attached to welder's shoulder.*	199.4		46.0		5.16	
Zinc-silicate	Oxy-acetylene	1′ above and 1′ back of welding.*	45.9		0.1		3.53	
" "	" "	3′ above and 2½′ back of welding.*	22.8		0.3		1.24	
" "	" "	3′ above and 2½′ back of welding.*	21.4		0.1		1.56	
" "	" "	3′ above and 2′ back of welding.*	18.0	26.81	0.2	0.26	1.80	1.96
" "	" "	3′ above and 2′ back of welding.*	42.6		0.3		1.80	
" "	" "	3′ above and 2′ back of welding.*	19.9		0.3		1.76	
" "	" "	3′ above and 2′ back of welding.*	17.1		0.5		2.00	
Galv. steel	Elect. arc	2′ above and 1′ back of welding.*	65.5		35.6		0.40	
" "	" "	2′ above welder's face.	152.0	108.5	25.3	18.35	0.69	0.52
" "	" "	6′ above the floor. 5′ in front of welder.	31.5		0.4		0.35	
" "	" "	Attached to welder's shoulders.*	185.0		12.1		0.64	
Galv. steel	Oxy-acetylene	2′ above and 2′ back of welding.*	106.6		0.8		0.66	
" "	" "	3′ above and 2½′ back of welding.*	33.0		1.4		0.24	
" "	" "	2′ above and 1′ back of welding.*	64.0	63.72	0.8	0.87	0.41	0.43
" "	" "	6′ above and 5′ back of welder.	30.0		0.5		0.30	
" "	" "	3′ above and 1′ back of welding.	85.0		0.8		0.55	
Clean steel	Elect. arc	2′ above and 1′ back of welding. (Control sample)	14.9		2.9		0	
" "	Oxy-acetylene‡	20′ from welding enclosure. (Room air. Control sample)	1.3		0.08		0	
" "	Elect. arc	20′ from welding enclosure. (Room air. Control sample)	0		0		0	

* Sample probe located near welder's face.
† Samples for Table I were not collected inside welder's hoods.
‡ 11 Fleetweld No. 5 welding rods consumed in 15 minutes.

TABLE II
Good Ventilation (Breathing Zone Samples)

Coating	Type weld	Location of sampling probe	Fume concentrations, mg/M³		
			ZnO	Fe_2O_3	Pb
Zinc-silicate	Oxy-acetylene cutting	Attached near welder's nose.*	—	—	0.18
Zinc-silicate	Electric arc beading	Inserted in welder's hood.*	9.84	0.32	0.08
Zinc-silicate	Electric arc welding	Inserted in welder's hood.*	19.81	5.51	0.14
Galvanized steel	Oxy-acetylene cutting	Attached near nose.*	12.28	Trace	0.01
Galvanized steel	Electric arc welding	Inserted in welder's hood.*	6.63	0.84	0.01

* Welder located upwind from welding.

TABLE III
Room Air Samples (Down Wind from Welder)
Electric Arc Welding on Zinc-Silicate Coating

Location of sample probe	Fume concentrations, mg/M³					
	ZnO	Avg.	Fe_2O_3	Avg.	Pb	Avg.
3′ downwind from the welder. 3′ from floor.	15.4	14.15	4.13	3.94	0.81	0.78
3′ downwind from the welder. 3′ from floor.	12.9		3.75		0.76	
20′ downwind from the welder. 3′ from floor.	4.9	5.86	0.26	0.69	0.26	0.25
20′ downwind from the welder. 3′ from floor.	6.8		1.13		0.24	
20′ downwind from the welder. 6′ from floor.	30.5	22.3	1.47	1.76	0.27	0.40
20′ downwind from the welder. 6′ from floor.	14.1		2.05		0.53	

under various conditions of ventilation in the welding shop. Within each table the results are subdivided to show the amounts of zinc oxide, iron oxide, and lead calculated as the element found in the air during welding by either oxy-acetylene or electric arc on the two types of coatings investigated (zinc-silicate and galvanizing).

Table I deals with samples of air collected while welding in an enclosure. The data show the zinc oxide and lead concentrations found when welding on zinc-silicate and galvanized coated steel in confined or poorly ventilated areas. Twenty-three samples were collected in the enclosure at distances of three to six feet from the welding. Several samples were taken near the welder's face and can be considered breathing zone samples although no samples shown in Table I were collected beneath his hood. Two control samples were taken 20 feet from the welding enclosure when the only welding in progress in the shop was on plain steel. Note that lead was nil on these samples. Another control sample was run to detect any contribution by the welding rods to the lead pick-up. Welding was done on clean steel while eleven Fleetweld No. 5 welding rods were consumed. Note that again the lead value is nil.

The data included in Table II illustrate the effectiveness of good ventilation in reducing lead and zinc fumes in the breathing zone of the welder. During electric arc welding on zinc-silicate coated material, the sample probe was inserted inside the hood of the welder who sat upwind from his work. Since no hood is worn during oxy-acetylene welding, a probe was held near the welder's nose. The samples for this test series were collected in a shop having high ceilings, open bays and exhaust fans to augment the good natural breeze that existed. Fumes drifted away from the welder at about one foot per second.

The data in Table III show the fumes present downwind from the welder. Three samples were

TABLE IV
Outdoor Samples (10-MPH Wind)

Coating	Type weld	Location of sampling probe	Fume concentrations, mg/M³		
			ZnO	Fe_2O_3	Pb
Zinc-silicate	Electric arc	Welder sat upwind. Probe inserted in hood.	4.22	0.29	0.06
Galvanized steel	Electric arc	Welder sat upwind. Probe inserted in hood.	13.24	0.55	0.01
Galvanized steel	Oxy-acetylene (cutting)	Welder sat upwind. Probe was held 3″ from nose.	2.40	0.09	0.00

collected simultaneously: one was taken three feet downwind from the welder and at the same time two samples were collected 20 feet downwind, three feet and six feet from the floor. An exhaust fan was moving the air away from the welding operation and toward the sampler at about one foot per second.

Table IV shows the effect of welding outdoors. There was a ten-mile-per-hour breeze while these samples were collected. The welder stood upwind from the work with the sample probe placed inside his hood. Eddy currents sometimes blew the fumes into his face but the lead recovered from these samples indicated tolerable conditions.

Conclusion

This investigation shows that welding on steel having a zinc-silicate coating and on galvanized steel can produce fumes containing lead and zinc in amounts exceeding the MAC's, and that zinc-silicate coated steel is a significant source of lead fume when welded by either oxy-acetylene or electric arc. In a confined area it was found that fumes contained lead concentrations four to seventy-five times the MAC, and zinc one to fourteen times the MAC. A greater degree of exposure occurs during electric arc welding than during torch welding partially because the welder, by necessity, must hold his face nearer his work.

Galvanized steel when welded in an enclosed area gave off fumes containing two to four times the MAC of lead and three to twelve times the MAC of zinc. The hazard, however, becomes almost negligible when the welding is carried out in reasonably well ventilated areas.

The need for good ventilation is demonstrated by the results of the investigation. Even during the summer months when natural ventilation was thought to be adequate, the MAC's for both lead and zinc were occasionally exceeded in the sampling area. In winter, when ventilation depended on exhaust fans, lead fumes two to four times the MAC occurred twenty feet downwind from the welder. Outdoors, in a 10-mph wind, satisfactory dilution of fumes is usually attained within one foot of the welding.

Recommendations

Recognition of the potential health hazard from fumes due to high temperature operations on zinc-silicate coated and galvanized steel should be followed by the necessary steps to prevent significant exposure. This is chiefly accomplished by proper ventilation.

Standard recommendations for ventilation are found in the American Standard Booklet Z-49.1 1958 *Safety in Welding and Cutting* published by the American Welding Society. Section 8 covers the ventilation problem in detail. Particular reference to zinc and lead are found in section 8.6 and 8.7, page 43.

Summary

An investigation was made to determine whether or not welding and cutting operations on steel with inorganic coatings consisting of zinc-silicate or galvanizing could give rise to hazardous amounts of lead and zinc fumes. Results of the investigation show that at the welder's breathing level such welding or cutting operations on zinc-silicate coated steel in poorly ventilated areas gave rise to fumes containing four to seventy-five times the maximum allowable concentrations of lead, and one to fourteen times the MAC of zinc. The same operations on galvanized steel under similar conditions produced fumes containing two to four times the MAC of lead, and three to twelve times the MAC of zinc. When ventilation was adequate the MAC was not greatly or consistently exceeded for either type of metal coating.

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Survey of Lead Hazard in Indoor Firing Ranges

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Introduction

AIRBORNE lead in firing ranges is generated in three ways: by combustion of the lead compounds usually found in priming compositions, by abrasion of the bullet when it leaves the gun (an amount which varies with gun condition and alignment of cylinders), and by fragmentation of the bullet when it strikes the target or backstop.[1, 2] Two lead poisoning incidents have been reported in the literature,[3, 4] one in a city police practice range, the other in a shooting gallery. Recent articles[1, 5] have called attention to the hazard in such ranges under conditions of poor ventilation.

Early in 1957 an instructor at an Ottawa firing range was found to be suffering from lead poisoning. This condition was discovered by chance when the instructor was turned down as a blood donor at a transfusion unit and referred by them to his physician for further investigation. A urine sample examined at that time was found to contain 1000 micrograms of lead per liter, about seven times what some authorities consider to be the threshold limit. A qualitative test on this sample also revealed the presence of coproporphyrin, another diagnostic sign in lead poisoning.

A urine sample from a second instructor in this range was then obtained and was found to contain 200 micrograms of lead per liter and a coproporphyrin test was strongly positive.

As a result of these findings an environmental survey of this range was carried out. The survey consisted of the measurement of air concentrations of lead during firing practice, analysis of settled dust samples for lead, and ventilation measurements. Airborne lead concentrations were found to be many times the amount recommended as acceptable for continuous industrial exposure, settled dust samples to have a substantial lead content and ventilation to be substandard.

Specific corrective measures were recommended to reduce lead exposure at this range and because of the very high airborne lead concentrations found it was also recommended that the survey be extended to other ranges. Over the past two years environmental studies of the lead hazard have been made at six ranges in different parts of Canada. In addition to these environmental studies, arrangements were made during the winter of 1958–59 to have urine samples submitted at monthly intervals from a few individuals firing regularly at each of the above and two additional ranges. These samples were analyzed for lead content only.

About one hundred air samples and one hundred and thirty urine samples were analyzed for lead. Lead-in-air concentrations measured were generally high, indicating a significant hazard to instructors and other personnel who are in the ranges for more than brief intervals. The lead content of urine samples was generally quite low, except for men working almost full time in the ranges.

Description of Firing Ranges

The firing ranges surveyed varied in length from 80 to 126 feet, in width from 20 to 36 feet and in height from 7½ to 12 feet. Five were located in basements; one on the top floor of a building which had been designed as a parking garage. Three had walls and ceilings covered with acoustical tile for noise control. All had some form of mechanical supply and/or exhaust ventilation.

Each range was equipped for simultaneous firing by four to eight men with rifles or revolvers. The revolvers used were .38 special or .22 calibre; the rifles, .22 calibre. The ammunition used with the .38 revolvers was known as .38 wadcutters.

For revolver practice the men stood in cubicles or at a table 20 yards from the target line. For rifle practice, the shooting was from a prone position 25 yards from the targets. Metal backstops were used at all ranges and spent shot usually fell into sandboxes. At one range the backstop was covered with a plywood shield. The targets in all cases were supported on an overhead wire and could be reeled in for inspection.

Three of the ranges were used for instructional purposes primarily and these had instructors on

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duty either full or a substantial part of the time. The other ranges were in intermittent use. Annual ammunition consumption at one training range amounted to 30,000 rounds of .38 calibre ammunition and 70,000 rounds of .22 calibre ammunition.

Survey Procedure

Two air sampling instruments were used during the survey, the Mine Safety Appliances Company electrostatic sampler and the Millipore Filter Corporation aerosol sampler. Both of these instruments are established as efficient air sampling devices for the collection of dust and fume. The electrostatic sampler samples air at the rate of 3 cfm and any particulate lead in the air is deposited on the inside of aluminum tubes 1½ inches in diameter. With the Millipore Filter apparatus the airborne particulates are deposited directly on the filter material. We used filters 2 inches in diameter and sampled air with this apparatus at a rate of 5–10 liters per minute. The Millipore Filter apparatus was found to be much more convenient to use.

Air samples were collected during various firing procedures and with different types of weapons and ammunition. All samples were collected at breathing level, many beside men while they were firing, others where the men gather for instructional purposes, a few in the target end of the range, and some in areas adjoining ranges. A number of samples were collected before and after firing activity. Collection of air samples is illustrated in Figure 1.

Settled dust samples were collected in one range only, as it was felt that this information was of limited utility. Airflow measurements were made with an Alnor swinging-vane anemometer at air supply and exhaust grilles and doorways.

Urine samples, about 200 ml each, were submitted in specially prepared polyethylene bottles, and it was requested that the samples be collected off the range on the first Friday of each month. Individuals were asked also to report the number of hours they spent in the ranges during the previous week and month and to state how much ammunition was consumed by themselves and their associates during those periods.

Lead analyses of air samples were made by a polarographic technique; urine samples were analyzed by a modification of one of the dithizone methods.

The First Environmental Survey

The initial range surveyed, in which the poisoning incident to which reference was made took place, was located in the sub-basement of a large office building. Its dimensions were 90 x 25 x 10

FIGURE 1. Collection of air sample with Millipore filter apparatus during firing practice. Man at the extreme left is placing cotton wad in his ear for protection against noise.

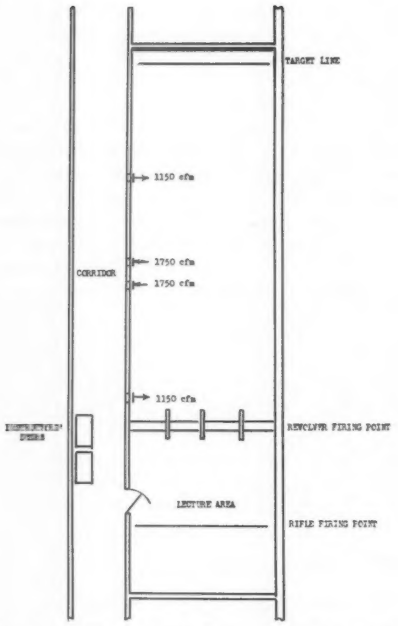

FIGURE 2. Plan of firing range where poisoning incident occurred.

feet and there was a single entrance door leading to a corridor. Figure 2 shows a plan view of this range.

This was a training range employing two full-time instructors. When not actually instructing in the range these two men worked at desks which were located just outside the entrance door.

The range was equipped with both supply and exhaust ventilation. At the time of the survey the exhaust volume amounted to 3500 cfm, the supply volume 2300 cfm. At the time of the poisoning incident, it was reported that the exhaust system had been partly closed off. With the exhaust system closed off to simulate these conditions, a very definite outward movement of air was noted through the door into the corridor.

Four sets of simultaneous air samples were collected. Three of these were taken with samplers located at the firing line and at the target line before firing, during firing with the ventilation system in full operation and during firing with the ventilation system as it was at the time of the poisoning incident. One pair of samples was collected after firing had ceased, with one sampler located in the centre of the range and the other at the instructor's desk in the corridor. Results of these tests are shown in Table I.

This series of tests revealed concentrations of lead 5 to 24 times the maximum acceptable concentration of 0.2 mg per cubic meter. High concentrations were recorded during firing both with the ventilation on full and as it was at the time of the poisoning incident. It had been suspected that samples might have been contaminated by large fragments of lead formed when the bullets struck the metal backstop. However, a very substantial lead concentration was found in the centre of the range after firing — some 35 times the maximum acceptable concentration and a lead concentration of over four times the threshold limit after firing in the vicinity of the instructor's desk. Also, air concentrations of lead near the backstop were not much different from those at the firing point.

Three settled dust samples were collected during this survey and analyzed for lead content. A sample collected from a pipe over the desk of one of the instructors in the corridor was found to contain 12.5% lead. A sample from the top of a cupboard in the range was found to contain 25% lead; a sample from one of the exhaust inlet grilles contained 17.5% lead. The high lead concentration in the dust from the exhaust grille was evidence that some lead was finding its way back into the general ventilation system of the building and being distributed to other areas.

As a result of the above findings it was recommended that the air supply and exhaust volumes be increased, that air supply inlets be relocated and that the connection between the exhaust system and the general ventilation system be severed.

Surveys of Other Training Ranges

The second range surveyed was also a training range. Sixteen air samples were collected for lead

TABLE I

Air Analysis for Lead—Range 1

Firing activity	Airborne lead—mg per M³				Ventilation
	Firing point	Target line	Centre of range	Desk in corridor	
None for 10 minutes before sample collected.	0.11	0.09			On full
Four men fired 20 rounds each with 38 revolvers	1.0	4.7			On full
Four men fired 20 rounds each with .38 revolvers	4.2	2.1			Restricted to conditions during poisoning incident
No firing for 6 minutes before sample started.			6.9	0.85	As above

determination; eight during the course of a normal training session, and eight during a special test which was arranged to measure the relative contamination at target and firing locations with and without exhaust fans in operation. The range differed from the first one in that the ceiling height was somewhat lower and the ventilation was somewhat inferior. It was ventilated by two wall-mounted exhaust fans which had a combined free air capacity of about 3000 cfm but no provision had been made for air supply and the entry door to the range had to be closed during firing because of the noise, so that ventilation was substantially reduced. Air concentrations of lead are shown in Table II. This series of tests again showed very substantial concentrations of lead during firing activity and after firing had ceased.

Shortly after this range was surveyed an instructor at one of the other ranges had a urine sample analyzed at a local hospital and was reported to be excreting lead at the rate of 450 micrograms per liter. A more extensive air analysis survey was carried out at this range and some 40 air samples were collected under various conditions of firing and other activities. This data is summarized in Table III.

TABLE II

Air Analysis for Lead—Range 2

No. samples	Firing activity	Airborne lead—mg per M³			Ventilation
		Firing point	Target line	Instructor's desk	
2	Four men fired 100 rds each with .38 revolvers	14.5 & 14.9			On
1	None—break period			4.1	On
2	Four men firing steadily with .38 revolvers	14.3 & 18.5		17.0	On
1	None. Sample started 5 mins. after firing ceased			1.4	On
2 simultaneous	Two men fired 50 rounds each with .38 revolvers	7.0	8.0		On
"	None. Sample started 2 mins. after firing ceased	3.8	2.2		On
"	Two men fired 50 rds. each with .38 revolvers	12.1	14.8		Off
"	None. Sample started 2 mins. after firing ceased	13.4	19.0		Off

TABLE III

Summary of Airborne Lead Concentrations—Range 3*

Operation	No. of samples	Airborne lead		Average divided by M.A.C.
		Range of values	Average	
Firing .22 rifles	6	0.042–0.22	0.11	0.55
Firing .22 revolvers	10	1.0–7.2	2.56	12.8
Firing .38 revolvers (training rate of fire)	6	0.54–30	18.4	90
Firing .38 revolvers (CPC rate of fire)	3	27–63	48	240
In armourer's shop adjoining range while firing .38 revolvers at training rate of fire	6	0.22–0.79	0.31	1.55
Sweeping floor	2	0.66 & 1.6		
Before firing commenced in morning	3	.008–.011		

* Canadian Police College

TABLE IV

Airborne Lead Concentrations—Ranges 4–6

No. samples	Range	Operation	Airborne lead mg per M³		Ventilation
			Range of values	Average	
4	4	During firing	1.8–9.2	5.4	Off
5	4	During firing	1.8–3.6	2.7	On
2	4	After firing	29.1 & 6.9		Off
2	4	After firing	0.46 & 0.51		On
6	5	During firing	0.6–20.4	5.4	On
2	5	During firing	3.7 & 2.9		Off
6	6	During firing	3.2–20.0	13.5	On

TABLE V

Analysis of Urine Samples for Lead

Range	Hours of exposure month	Lead micrograms per liter						
		Nov	Dec	Jan	Feb	Mar	Apr	May
1	160	nil	nil	37	62	128	71	—
2	0– 12	26	29	13	17	45	36	15
3	5– 12	—	30	34	27	—	—	—
4	7– 30	—	37	19	52	18	18	18
5	3– 7	5	58	34	28	30	38	40
6	120–164	94	102	172	164	125	110	72
7	8– 10	—	16	52	39	21	22	—
8	0– 18	—	—	29	20	33	24	62
9	1– 10	—	—	—	—	—	36	62

Again very substantial concentrations of lead were recorded in the breathing zone of men engaged in firing. The data appeared to indicate a considerable variation in the lead output under different conditions of firing, most severe concentrations being recorded with .38 revolvers, lower concentrations with .22 revolvers and the lowest

with .22 rifles. Also of interest in this series was the substantial contamination of an adjoining shop.

Surveys of Practice Ranges

Three ranges which were used primarily for practice firing rather than for instructional purposes were included in the survey. These ranges were in use for only a few hours per week but in at least one range children were given firing instruction. All tests in these ranges were made during firing of .38 revolvers. Data is summarized in Table IV.

Analysis of Urine Samples for Lead

A selection of the urinalysis survey results from each range are recorded in Table V, together with an estimate of the time the various individuals were in the firing ranges. All of the lead in urine results were summarized by hours of exposure during the previous week, hours of exposure during the previous month, exposure to rounds fired during the previous week and exposure to rounds fired during the previous month. Average lead results varied from 29 to 42 micrograms except for men exposed more than 30 hours per week (76), 120 hours per month (97), more than 1,000 rounds of ammunition per week (56), or more than 5,000 rounds of ammunition per month (72).

According to Schrenk,[6] the normal urinary lead excretion varies from 10 to 80 micrograms per liter in large samples of a liter or more; and from 5 to 120 micrograms per liter in spot samples of 50 ml or more. Schrenk states that the mean value is in the order of 30 micrograms per liter and that values of 150–200 micrograms per liter have generally been used as a maximum safe limit. From an inspection of Table V it will be seen that only one individual was found to be excreting more than 150 micrograms of lead per liter during this survey and that only one other individual approached this limit. Most of the urine sample results showed lead in very much lower concentrations.

From the high lead-in-air concentrations which have been reported above it might have been expected that urine lead samples would have shown more evidence of lead absorption. We at first thought that the low urines were an indication that air samples contained many large size particles which could not be inhaled. However, the high concentrations of lead which we found after firing had ceased, would seem to indicate that this was not the case. Another possible explanation is that the lead is in a form which cannot be readily taken into the body. We rather doubt this as we would expect it to be in the form of a fine fume. Deposits on a few Millipore filter samples examined by microscope were found to consist largely of particles below one micron in size. A third explanation is that it might be related to the fact that exposure in most cases is discontinuous. Kehoe, in a recent paper,[7] states that when the respiratory exposure is discontinuous the individual reaches a plateau with respect to his rate of urinary excretion. Most of the individuals from whom lead samples were obtained had a very discontinuous exposure.

Control of the Lead Hazard

While the urinalysis survey did not indicate any substantial degree of lead absorption the fact that over the past two years three individuals in this group of firing ranges have shown excessive lead absorption, and at the same time substantial air concentrations of lead have been recorded, indicates that the exposure should be controlled.

The most important control measure is a good ventilation system. For individual ranges, we have recommended the supply of tempered fresh air with some form of draftless distribution over the firing end and exhaust ventilation from the target end of the range. Our aim has been essentially to flood the occupied end of the range with fresh air and we have recommended supply and exhaust volumes sufficient to maintain a linear flow towards the targets of at least 25 feet per minute. We have not attempted to restrict the cross-section of the range as has been suggested by other investigators.[1] This certainly has the advantage of greatly reducing the air volumes required but imposes some restrictions in the use of the range. Larger volumes are also desirable to aid better visibility during periods of considerable firing activity.

In addition to good ventilation firing ranges should maintain good housekeeping standards in order to prevent contamination caused by redispersal of settled dust.

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Myocological Contaminaton of Impinger Dust Samples

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STANDARD practice for the preparation of impinger tubes to be used for dust sampling dictates thorough washing and rinsing, followed by filling with the proper quantity of liquid entrapment medium and sealing until ready for use.

For the collection of mineral dusts, distilled water is quite often used as the collection medium. The tubes are generally prepared just prior to the time of collection, although in theory it may be argued that this is unnecessary as they are sealed immediately after filling.

Case Narrative

In preparation for a dust study, ten Greenburg-Smith impingers (GSI) and two midget impingers (MI) were prepared according to the method described above. Filling was accomplished from a five-gallon settled source of nonsterile distilled water via a polyethylene siphon tube. Two additional MI tubes which had been prepared previously from the same water source were also taken along.

In the field, calibrated Willson pumps were utilized for the GSI samples, while hand-operated pumps were used for the MI samples. The impinger tubes were unsealed only during the sampling operation, after which they were again sealed until aliquots were removed for counting.

In the laboratory, aliquots were removed from the MI tubes and plated into Dunn cells. Examination was by direct lightfield microscopy at 100×. The field observed was cluttered with numerous spherical objects approximately two microns in diameter (see Figure 1). Dilutions of 100 to 1 failed to reduce the number of particles per field to a level that could be easily counted.

Examination of samples from GSI's revealed a greatly lowered particle density marked by the absence of the spherical particles. This fact was strange in that some of the GSI and MI samples had been taken simultaneously. Microscopic examination of a control MI tube not used for sampling but taken into the field revealed the presence of vast numbers of the aforementioned spheroids. The converse was true for the GSI tubes. It was concluded that some outside contaminant had been somehow introduced into the MI but not into the GSI samples. This conclusion was strengthened by the fact that both types of tubes were filled from the same water source and this source was not found to contain the spheroids when specimens were examined microscopically.

Determination of the Nature of the Contaminant

Four hypotheses were proposed to explain the presence of the contaminant. These were as follows:

1. Dust remaining in the MI tubes due to inadequate washing prior to preparation.
2. Dust or like material introduced into the MI accidentally or deliberately.
3. Bacterial contamination (cocci).
4. Mycological contamination (spores).

That the objects resembled dust there was no doubt; several persons familiar with dust sampling and counting procedures readily asserted that the spheroids were dust particles. Under higher magnification (300×) the particles still appeared to be perfect spheres with crenated edges, exactly the same size. As it is extremely difficult (if not altogether impossible) to obtain such uniform dust particle size, hypothese 1 and 2 were tentatively rejected.

Hypothesis 3 was tested by cultures made from the MI and GSI tubes on both nutrient agar and blood agar; incubated overnight at 37°C. All cultures yielded only gram negative rods, thus tending to disprove this hypothesis. In addition, the size of these particles (approximately two microns in diameter) is greater than that of most cocci.[1]

Hypothesis 4 was then tested by culturing the water from the tubes on Sabouraud's medium. Mixed mold growth was obtained from the MI cultures, supporting this hypothesis.

Source of Contamination

Once the nature of the contaminant had been ascertained, the next step was to determine the source. Cultures on Sabouraud's medium were made of the water source, the laboratory air, the dust counting equipment, storage area, and other

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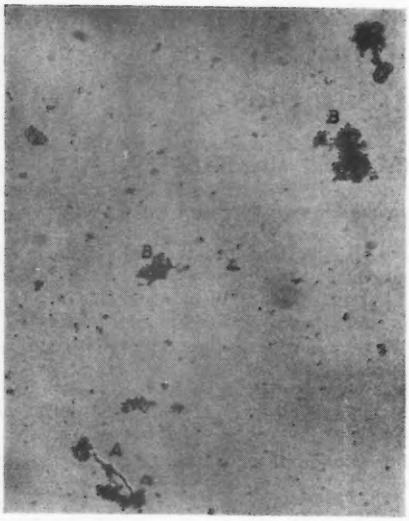

FIGURE 1. Photomicrograph of midget impinger contents at 100×. (Negative enlarged 2×.) Note mycelium at A and clumping at B. Clumping became more prevalent as sample aged; original examination revealed very few clumps.

potential sources of contamination. The only culture showing growth was taken from the rubber stopper used to seal the MI and to hold the glass nozzle in place (see Figure 2).

Discussion

Mold spore contamination of dust samples may not be rare, but reports of this do not appear in the literature. Control of this factor is important if accurate dust counts are to be obtained. If the mold is drawn into the tube during sampling and a small amount of organic material is present, the mold may grow and sporulate during the period between sample preparation and laboratory analysis.

Control of contamination by mold spores can be afforded by scrupulously clean sampling tubes along with a fungicidal agent in the distilled water, as some molds can utilize very minute quantities of organic materials for anabolism, perhaps even bits of cork or rubber stopper. The use of propyl or iso-propyl alcohol as the sampling medium should eliminate growth and subsequent sporulation. These liquids may be superior to distilled water in other respects also, as pointed out by Drinker and Hatch.[2]

FIGURE 2. Culture made from midget impinger stopper on Sabouraud's medium showing profuse mold growth.

Summary

Dust samples collected in a foundry were found to be contaminated with uniform spherical particles approximately two microns in diameter. Examination of the sample indicated that the contaminant was not dust but mold spores. These molds were found to be introduced from the rubber stoppers of the midget impinger tubes. The use of scrupulously clean glassware and propyl or iso-propyl alcohol as the sampling medium should eliminate the possibility of mold contamination.

Acknowledgment

Acknowledgment is hereby made of the efforts of Parviz Charlang who prepared the biological cultures, Jack C. Rogers and Raymond A. Miller for their helpful comments and Bernard V. Soibelman for the photography. All biological procedures were carried out by the Division of Laboratories, Los Angeles City Health Department, Charles F. Pait, M.D., Director.

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Use of Spider Silk in Aerosol Research

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MUCH information about the characteristics of aerosols is obtained from studies of the individual particles which make up the aerosol. Evaporation studies, for instance, are often made of single drops of liquid supported on fine fibers or filaments.

The size of the fiber is of major importance in this work, since the effect of the fiber is to elongate and distort the spherical shape of the drop. The smaller the diameter of the fiber, the less distortion of the drop, and the smaller the diameter of the drop which can be examined. Droplets as small as 70 microns in diameter have been studied by this method, using fibers six microns in diameter.

The purpose of this memorandum is to describe an extension of the fiber technique. In recent work of this group, it was found that single fibers less than 0.2 microns in diameter (half the wave length of visible light), are available in the form of spider silk of a certain family of widely-occurring spiders. The insect which produces these fibers belongs to the comb-footed spider family Theridiidae. The adult is about 2 mm in diameter. It is to be found in wooden debris in back-yards and gardens.

FIGURE 1. Glass collar used in mounting fiber.

In order to obtain the fiber as a filament and in a dust-free condition, the spider is picked up on a twig or wooden dowel and, by mechanical manipulation, induced to drop from its support. This it does by fastening a strand of silk to the support and dropping by the strand as it is extruded. The silk may be wound on a miniature reel as it is formed, or, for microscope work, stretched across glass collars 5 mm high and 10 mm inside diameter and cemented in place with collodion (Figure 1).

One of the best ways of placing aerosol particles upon a spider silk mount is to set up the aerosol of interest in a small chamber. If a small port (about 1 mm diameter) in the bottom of the chamber is opened, a stream of particles will issue from it. This stream may be directed selectively onto the fiber mounted on the glass collar as described above, without contaminating the support with aerosol. The number of particles sticking to the fiber is a function of the exposure time. Experience will dictate the exposure time required to obtain just a few droplets on the entire fiber. Unwanted droplets may be removed mechanically, using fine wire.

Microscopic droplets suspended as described above may be used in shock tubes in studying the mechanics of atomization, in special containers made of porous barriers for evaporation studies in still air, and in special microscope mounts for determining boundary layer phenomena and wind effects on evaporating drops. The technique may be used also in studies of droplets in electrical fields, such as exist in the

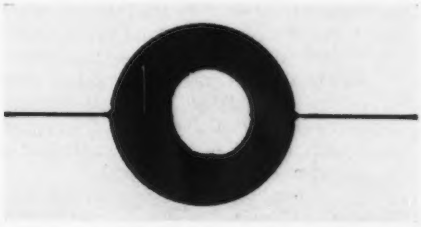

FIGURE 2. Photomicrograph of hexadecane droplet upon a submicron fiber of spider silk.

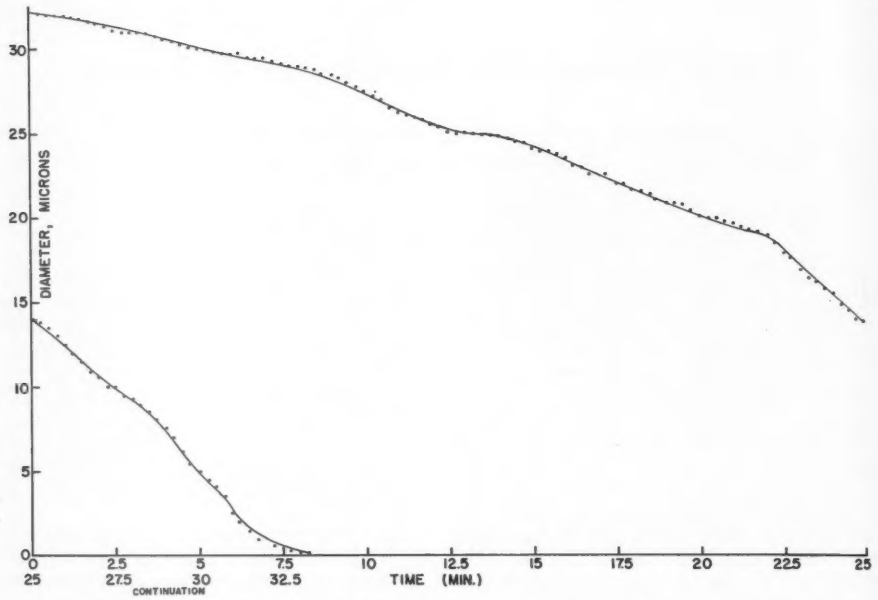

FIGURE 3. Time relationship for hexadecane droplet evaporating from an ultra-fine fiber.

Millikan oil drop apparatus, and in studies of the mechanics of aerosol filtration.

Figure 2 shows a photomicrograph of an evaporating droplet on a submicron fiber, while Figure 3 is a graph made from data gathered using the ultra-fine fiber technique.

As a result of the improved method for suspending micro droplets from ultra fine fibers, new and useful information may be gathered from the direct observation of single microscopic liquid particles under conditions almost equivalent to free suspension in air

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NEW PUBLICATIONS BY ACGIH

A Manual of Air Sampling Instruments for Evaluation of Atmospheric Contaminants was published recently by the Committee on Air Sampling Instruments of the American Conference of Governmental Industrial Hygienists. This manual contains descriptions of more than 100 instruments and equipment generally used in the field of industrial hygiene. This publication is a revision of Section I of the Encyclopedia of Instrumentation for Industrial Hygiene published in 1956 by the University of Michigan's Institute of Industrial Health. About one-half of the instruments in the new Manual did not appear in Section I, and the data on many others has been revised to present the latest available information. A loose-leaf format was adopted for the new Manual so that future revisions may be facilitated.

The ACGIH has also released another publication, *Guide to Records for Health Services in Small Industries*. The types of records which should be kept in employee health services of small industrial or commercial establishments are discussed with examples of the basic forms.

These publications are available from the Secretary-Treasurer, American Conference of Governmental Industrial Hygienists, 1014 Broadway, Cincinnati 2, Ohio. The price of the Manual of Air Sampling Instruments is $7.50; the Guide to Records costs $1.00 per copy.

HYGIENIC GUIDE SERIES

Acetonitrile

(Methyl Cyanide, Ethanenitrile)

I. Hygienic Standards

A. RECOMMENDED MAXIMUM ATMOSPHERIC CONCENTRATION (8 hours): No official value has been suggested. Forty parts of vapor per million parts of air (ppm), by volume, has been suggested on the basis of extensive animal experiments and limited data on human subjects.[1]

B. SEVERITY OF HAZARDS:

1. *Health:* Moderate or severe for acute or subacute exposures, depending on concentrations encountered. High concentrations such as exist in tanks or in closed spaces can cause a delayed onset (several hours or more) of weakness, nausea, vomiting, chest or abdominal pain, vascular changes, shock and death. The levels causing such effects are unknown but are probably over 500 ppm. Elevated blood CN^- or SCN^- levels have been found in such cases. The liquid or concentrated water solutions readily penetrate rabbit skin, and presumably also present a hazard to humans by the same route.[1, 2]
2. *Fire and Explosion:*
   Flash point: 42°F (open cup).

C. SHORT EXPOSURE TOLERANCE: Two human subjects inhaled 160 ppm for four hours, and one subject experienced a slight flushing of the face two hours later, and a slight feeling of bronchial tightness five hours later. A week prior to this, the same two subjects inhaled 80 ppm with no effects.[1] Humans accidentally inhaling 500 ppm for brief periods reported some irritation of nose and throat. A high incidence of fatalities in rats, dogs, guinea pigs occurred at 16,000 ppm for four hours. Some deaths resulted at 4000 ppm and none at 1000 ppm.

D. ATMOSPHERIC CONCENTRATION IMMEDIATELY HAZARDOUS TO LIFE: Not known for humans, but 53,000 ppm killed rats in 15 minutes.[1] Amdur reported the death of one worker after a continuous exposure of about 12 hours to an unknown but probably high concentration inside a tank.[2] Several other exposed workers had moderate to severe symptoms.

II. Significant Properties

Colorless liquid with a distinctive odor

Chemical formula:	CH_3CN
Molecular weight:	41.05
Specific gravity:	0.7839 (20°/20°C)
Boiling point:	81.6°C
Vapor density:	1.41 (air = 1)
Vapor pressure:	73 mm at 20°C

Air saturated at 20°C. and 760 mm Hg contains approximately 96,000 ppm

Solubility: Infinitely miscible in water

At 25°C and 760 mm Hg:

1 ppm of vapor:	0.001679 mg/liter
1 mg/liter:	595.3 ppm

III. Industrial Hygiene Practice

A. RECOGNITION: Three human subjects detected the odor at 40 ppm. Olfactory fatigue began in two to three hours.[1] Acetonitrile is used in closed equipment as synthetic fiber spinning solvent, and its high solvency suggests it may be encountered where many unusual solvent problems must be met.

B. EVALUATION OF EXPOSURE:

1. *Direct Instrumentation:* None available.
2. *Chemical:* Adaptation of the method described by Jacobs for the determination of acrylonitrile.[1, 3]

C. RECOMMENDED CONTROL PROCEDURES: Enclosure of processes accompanied by local or general ventilation. Education of

all exposed persons regarding the hazard and first aid measures.

IV. Specific Procedures

A. FIRST AID: Remove to an uncontaminated atmosphere and summon medical aid. Start artificial respiration if breathing has ceased. Remove contaminated clothing and wash affected skin areas with soap and water. Rinse out eyes, if affected, with copious quatities of water for at least 15 minutes.

B. SPECIFIC MEDICAL PROCEDURES: The effectiveness of the usual specific medical antidotes[4, 5] for cyanide and other nitriles is uncertain. In two of the more severe cases seen by Amdur, the therapy was essentially supportive and consisted of the use of oxygen, fluids and whole blood intravenously, ascorbic acid and sodium thiosulfate. In the other cases, no special therapy seemed necessary. Amdur[6] feels that, because the clinical picture is accompanied by rather marked disturbance of the vascular system with a shock-like state, the use of agents such as amyl-nitrite or sodium nitrite which dilate blood vessels is contraindicated. Further investigation of the specific therapy of acetonitrile poisoning is needed.

While blood or urinary thiocyanate levels may be elevated in cases of acetonitrile exposure, they are of little value as an index of the adequacy of the control measures under ordinary conditions of exposure.

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Diethylamine

I. Hygienic Standards

A. RECOMMENDED MAXIMUM ATMOSPHERIC CONCENTRATION (8 hours): 25 parts of vapor per million parts of air, by volume (ppm).[1]

1. *Basis for Recommendation:* Toxicological observations on animals.[3, 4]

B. SEVERITY OF HAZARDS:

1. *Health:* Severe for acute and moderate for chronic exposures. The effects are primary irritation of mucous membranes, particularly of the eyes, and skin. Liquid contact with eyes causes very severe damage; causes skin blistering and necrosis of the skin. Penetration of the skin is rapid.

C. SHORT EXPOSURE TOLERANCE: Not known.

D. ATMOSPHERIC CONCENTRATION IMMEDIATELY HAZARDOUS TO LIFE: Unknown for human. Saturated vapor for five minutes killed all rats.[3] Concentrations dangerous to life will not be tolerated voluntarily.

II. Significant Properties

Chemical formula:	$(CH_3CH_2)_2NH$
Molecular weight:	73.14
Specific gravity:	0.7062 20/20°C
Boiling point:	55.5°C, 760 mm
Freezing point:	−49.8°C
Vapor pressure:	195 mm Hg at 20°C
Solubility:	Completely in water
At 25°C and 760 mm Hg:	
1 ppm of vapor:	0.00299 mg/liter
1 mg/liter:	334 ppm

III. Industrial Hygiene Practice

A. RECOGNITION: By its ammoniacal odor and irritation of mucous membranes. Used in manufacture of accelerators, insecticides, emulsifiers, and pharmaceuticals.

B. EVALUATION OF EXPOSURES:

1. *Chemical Method:* Can be determined by variations of the micro-Kjeldahl method.[2]

C. RECOMMENDED CONTROL PROCEDURES: Maintain concentration below 25 ppm by general ventilation or enclosure of process. Personal protective equipment against skin or eye splashes is required. Convenient facilities in work areas for irrigating eye or skin should be provided.

IV. Specific Procedures

A. FIRST AID: Remove to a non-contaminated area. If the material has contacted the skin, affected clothing should be removed at once and surfaces flushed with water for 10 min. First aid treatment for eye contact is immediate and prolonged washing with water for at least 10 minutes, followed by prompt care by a physician.

B. SPECIAL MEDICAL PROCEDURES: In preplacement, consider carefully the possible aggravation of chronic pulmonary conditions, monocular vision and pre-existing dermatitis. Follow-up examinations should include a search for eye symptoms or changes, especially if exposures are continuous and above the threshold limit.

V. References

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Nitropropane

The two isomeric forms, 1-nitropropane and 2-nitropropane are similar in toxicological and physical properties.

I. Hygienic Standards

A. RECOMMENDED MAXIMUM ATMOSPHERIC CONCENTRATIONS (8 hours): 2-Nitropropane 50 parts per million parts of air, by volume (ppm).[1] No official or tentative value has been stated for 1-nitropropane. Until more information is available, it seems desirable to keep levels well below the maximum limits for 2-nitropropane.

1. *Basis for Recommendation* of value for 2-nitropropane: Limited observation of effects in humans and animal experiments.[2, 3, 5a] Symptoms may occur in some humans at 50 ppm.

B. SEVERITY OF HAZARDS:

1. *Health:* Moderate for both acute and chronic exposures. High concentrations of 2-nitropropane produced dyspnea, cyanosis, coma and death in animals. The pathologic changes included pulmonary edema and necrosis of the liver.[3] Methemoglobinemia was present in cats and rabbits. Prolonged exposure to about 85 ppm produced slight degenerative changes in the organs of some animals and caused the appearance of Heinz bodies in the blood. Earlier work had indicated 1-nitropropane to be somewhat more toxic than 2-nitropropane.[4] No skin injury or absorption was noted in animals.

   Workers exposed to 2-nitropropane in concentrations ranging from 30 ppm to 300 ppm reported symptoms of headache, dizziness, nausea, vomiting and diarrhea.[2, 5] Some complaints of respiratory tract irritation were also noted.

2. *Fire:* Moderate fire and explosion hazard. Flash points for 1- and 2-nitropropane are 120°F and 103°F respectively (open cups). The lower limit of flammability in air is 2.6%. Under some conditions salts, which are explosive when dry, are formed by reaction of inorganic bases with nitroparaffins.[6]

C. SHORT EXPOSURE TOLERANCE: Animals survived single exposures to concentrations of 500–1000 ppm for periods up to one hour without serious effects.[3]

D. ATMOSPHERIC CONCENTRATION IMMEDIATELY HAZARDOUS TO LIFE: 5000 ppm of 1-nitropropane and 3000–9000 ppm of 2-nitropropane were fatal to animals in relatively brief periods.[3, 4]

II. Significant Properties

	1-nitropropane	*2-nitropropane*
Chemical formula:	$CH_3CH_2CH_2\text{-}NO_2$	$CH_3CHNO_2\text{-}CH_3$
Molecular weight:	89.09	89.09
Specific gravity: 20°/4°C	1.001	0.990
Boiling point:	132°C	120°C
Vapor pressure:	13 mm (25°C)	20 mm (25°C)
Solubility:	Slightly soluble in water; soluble in most organic solvents.	
At 25°C and 760 mm Hg:		
1 ppm of vapor:	0.0036 mg/liter	
1 mg/liter:	275 ppm	

III. Industrial Hygiene Practice

A. RECOGNITION: Used chiefly as solvent for special lacquers and dopes. The odor is detectable at 300 ppm but not at 80 ppm.[3] The lack of warning properties makes it necessary to check all areas where it is used by air analyses.

B. EVALUATION OF EXPOSURES:
1. *Instrumentation:* Combustible gas indicator.
2. *Chemical:* Collection on silica gel, in appropriate solvent, or in sulfuric acid, followed by colorimetric determination. Methods specific for primary and secondary nitroparaffins are available.[7, 8, 9]

C. RECOMMENDED CONTROL PROCEDURES: Maintain atmospheric concentrations of nitropropane below 50 ppm. Spraying of lacquers containing nitropropane should only be done in a well ventilated spray booth. If high concentrations of vapor are encountered, a supplied air respirator should be used, since the unusually high heat of adsorption on activated charcoal may create a problem if chemical cartridge respirators are used.[6]

IV. Specific Procedures

A. FIRST AID: In case of pulmonary symptoms or cyanosis, remove workers from contaminated area at once, place at bed rest, use oxygen if respiratory distress is present and obtain medical attention.

B. SPECIAL MEDICAL PROCEDURES: Persons exposed to concentrations approaching or exceeding the MAC should have careful periodic medical evaluations, with special emphasis on examination of red cells for Heinz bodies, methemoglobin determinations and liver function tests.

V. References

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Diethylene Triamine

(2,2′-Diaminodiethylamine)

I. Hygienic Standards

A. RECOMMENDED MAXIMUM ATMOSPHERIC CONCENTRATION (8 hours): Not known. Because of tendency to skin and pulmonary sensitization, all exposures must be reduced to the lowest possible level.

B. SEVERITY OF HAZARDS:
1. *Health:* Severe for both acute and chronic exposures. Liquid contact can cause severe skin and eye damage. Pulmonary[1] and cutaneous sensitization occurs with relatively high frequency unless adequate measures are taken to control all contacts. Remarkably little exposure is necessary in some cases to induce a high degree of sensitivity. The mechanism of action is probably a cross linking of proteins with the bifunctional amino groups.
2. *Fire:* Low. Flash point 102°C (open cup).

C. SHORT EXPOSURE TOLERANCE: Unknown. There were no animal deaths following an 8-hour exposure to saturated vapors at room temperature.[4]

D. ATMOSPHERIC CONCENTRATION IMMEDIATELY HAZARDOUS TO LIFE: Unknown.

II. Significant Properties

Diethylene triamine is a yellow liquid with a strong ammoniacal odor.

Chemical formula: $NH_2CH_2CH_2NHCH_2$
CH_2NH_2

Molecular weight:	103.17
Specific gravity:	0.951 20°C/20°C
Boiling point:	207.1°C (760 mm)
Relative density:	3.48 (air = 1)
Vapor pressure:	0.37 mm Hg at 20°C 22 mm Hg at 103°C
Solubility:	Completely in water
At 25°C and 760 mm Hg:	
1 ppm of vapor:	0.0042 mg/liter
1 mg/liter:	237 ppm

III. Industrial Hygiene Practice

A. RECOGNITION: Used as a catalyst for epoxy resins, an intermediate for textile finishing agents, and an intermediate in the manufacture of pharmaceuticals and nitrogen-containing compounds. It may be identified by its odor, but the sense of smell fatigues rapidly and this is an unreliable guide to safe levels. If a definite odor of diethylene triamine can be detected, the controls are inadequate.

B. EVALUATION OF EXPOSURES:

1. *Chemical:* May be determined by a Kjeldahl method.[2] Grandjean[5] describes a method for triethylene tetramine which may be applicable. The sampling medium was either 0.1*N* HCl, or cation and CO_2 free distilled water. A color was formed with 1,2-naphthoquinone 4-sulfonic acid according to the method of Englis and Fiess.[6]

C. RECOMMENDED CONTROL PROCEDURES: A definite well organized program for control of the hazard is necessary in all uses of this material, even those involving relatively small quantities. This is particularly true in situations where repeated skin contact and inhalation may be encountered, as in its use as a curing agent or catalyst in epoxy resin systems. Other discussions of precautionary measures are found in the AIHA Hygienic Guide series, Epoxy Resin Systems, published in the *AIHA Journal 19:* 444 (1958)[7] and the Manufacturing Chemists' Association's *Chemical Safety Data Sheet SD-76* (1959).[3]

Skin contact should be avoided by the use of gloves, aprons, and clean clothing. Convenient hand washing facilities should be available in all areas where the amine is being used. Acid type hand cleaners are probably relatively effective in helping to remove this material from the skin. Provision should be made for convenient facilities in the work area for flushing the skin and eye. Disposable paper towels should be used in such areas. Proper facilities for clothes change and showers should be available.

Special housekeeping provisions are important, such as isolation of the areas where the material is used from other operations or areas where there is personnel traffic, decontamination of all equipment after use, special receptacles for collections of waste towels or other materials contaminated with the amine, and lining of any work surfaces with paper for easier cleaning of contaminated areas.

Some form of local exhaust ventilation is practically always necessary where diethylene triamine is to be handled. This should be supplemented by good general ventilation. All hoods should be checked to see that they are functioning properly, and supplies of the amine should be stored in well ventilated areas.

IV. Specific Procedures

A. FIRST AID: In the case of a splash of the liquid on the skin, any contaminated clothing should be removed at once and the underlying surface flushed with copious amounts of water for at least 10 minutes. This should be followed by thorough cleansing of the area with soap and water. In the case of eye contact, the eye should be immediately flushed with copious amounts of water for at least 15 minutes with the lids held open to assure adequate contact of water with the cornea. Immediate medical care should be obtained in the case of any eye splash. If an asthmatic attack occurs, the individual should be removed from the area at once, placed on bed rest with the head elevated, and medical attention obtained. Oxygen may be used if indicated.

B. SPECIAL MEDICAL PROCEDURES: Careful preplacement examinations should be made to determine pre-existing chronic pulmonary, asthmatic, or skin conditions. Careful medical follow-up examinations should be made of all exposed individuals. Withdrawal from all further exposure is occasionally necessary in instances where a high degree of sensitization results. In the event of contact dermatitis, an effort should be made to obtain proper medical treatment at the earliest possible moment and to discourage any attempts at self-treatment.

V. References

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5. GRANDJEAN, E.: *Brit. Jour. Ind. Med. 14:* 1 (1957).
6. ENGLIS, D. T., AND H. A. FIESS: *Ind. Eng. Chem. 36:* 604 (1944).
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Tetrachloroethylene

(Perchlorethylene)

I. Hygienic Standards

A. RECOMMENDED MAXIMUM ATMOSPHERIC CONCENTRATION (8 hours): 200 parts of vapor per million parts of air, by volume (ppm).[1] This level is believed to represent a maximum, which should not be exceeded in repeated daily exposure; it is not to be used as an integrated average concentration. A level of 100 ppm would be more suitable for the latter.

1. *Basis for Recommendation:* Human experience and animal experiments.[2]

B. SEVERITY OF HAZARDS:

1. *Health:* Moderate. Has been widely used with relatively few instances of serious illnesses or death reported. Narcotic effects may be observed at concentrations in excess of 200 ppm. Permanent injury to the nervous system, and a fatal case with symptoms of pulmonary edema and stasis with generalized edema have been reported from a high exposure to tetrachloroethylene.[3] Probable injury of the liver occurred in men operating degreasers using tetrachloroethylene.[4, 5]
2. *Fire:* Nonflammable. Products of decomposition found in the presence of flames or hot surfaces are highly irritating to the eyes and respiratory tract and cause metal corrosion.

C. SHORT EXPOSURE TOLERANCE: Human subjects developed signs of narcosis in minutes after exposure to 1000 ppm[6] (45 min) and 600 ppm.[2]

D. ATMOSPHERIC CONCENTRATION IMMEDIATELY HAZARDOUS TO LIFE: 6000 ppm produced unconsciousness in animals within a few minutes.[2]

II. Significant Properties

Tetrachloroethylene is a clear, colorless, nonflammable liquid with a distinctive, somewhat ethereal odor.

Chemical formula:	$Cl_2C{=}CCl_2$
Molecular weight:	165.83
Specific gravity:	1.62 at 20°C/4°C
Boiling point:	121.2°C
Vapor pressure:	19 mm. (25°C)
Vapor density:	5.7 (air = 1)
Density Saturated Vapor:	1.18 (air = 1)
Solubility:	Soluble in most organic liquids. Insoluble in water.
At 25°C and 760 mm:	
1 ppm	0.0068 mg/liter
1 mg/liter	148 ppm

III. Industrial Hygiene Practice

A. RECOGNITION: Used widely as a dry cleaning solvent, and in degreasers, and as a component of "safety" solvents, especially where fast evaporation is not needed; also used medically as an anthelmintic. 50 ppm is detectable by odor; 1000 ppm is irritating to mucous membranes in subjects who are not regularly working with the material.

B. EVALUATION OF EXPOSURES:

1. *Instrumentation:* Davis Halide meter or other instrument for halogenated hydrocarbons; interferometer; mass spectrometry.
2. *Chemical:* Collection on silica gel:
   (a) with subsequent hydrolysis with metallic sodium and determination of halide content.[7]
   (b) with expulsion from silica gel by heat, followed by combustion, trapping the chloride in sodium

carbonate-sodium formate solution and titration of the trapped chloride.[8]

(c) direct combustion followed by chloride titration as in (b) above.

A number of other chemical methods are described.[9, 10, 11, 12]

C. RECOMMENDED CONTROL PROCEDURES: Maintain air concentrations well below 200 ppm at all times in workroom atmosphere. The time weighted average concentration should probably not exceed 100 ppm. Frequently, good general ventilation will be adequate, especially if the solvent is not heated. When used in a degreaser, proper operating practices are important. Tetrachloroethylene vapor should not be permitted around open flames or very hot surfaces. Welding should be avoided in the presence of the vapor.

IV. Specific Procedures

A. FIRST AID: Remove from exposure any worker who shows dizziness, giddiness or sleepiness suggestive of early narcosis, put at bed rest and call a physician. If unconscious, artificial respiration and oxygen may be needed. The use of adrenalin should be avoided in any case of anesthesia with chlorinated solvents, because of possible ventricular fibrillation.

B. SPECIAL MEDICAL PROCEDURES: As in the case of other chlorinated solvents, persons with definite liver, renal, cardiac or neurologic disease should not be placed at work where a hazard of major acute exposure exists, or where there may be substantial repeated exposures. Careful periodic medical examinations should be made of persons regularly exposed to average concentrations of more than 100 ppm.

V. References

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ANACHEM CONFERENCE

THE EIGHTH DETROIT ANACHEM CONFERENCE will be held October 24, 25, and 26, 1960 at the McGregor Memorial Conference Center, Wayne State University, Detroit, Michigan. This year will see a program of increased scope which will present a "bill of fare" consisting of two categories: (1) papers reviewing methods and techniques that may find immediate application in the laboratory, and (2) papers devoted to new approaches to analytical chemistry. The subject matter will be related to polarography, spectroscopy, x-ray fluorescence, flame photometry, chromatography, absorption photometry, activation analysis, and other techniques as well as general chemical analysis.

Dr. Harry A. Bright of the National Bureau of Standards will receive the 1960 Anachem Award and give the Award Address.

For additional information write to M. D. Cooper, Chemistry Department, Research Laboratories, General Motors Corporation, 12 Mile and Mound Roads, Warren, Michigan.

News of Local Sections

Gulf Coast Section

New officers in this Section are Mr. O'Neil Banks, President; E. R. Hermann, President-Elect; and M. M. Sullivan, Secretary-Treasurer. At a recent meeting Mr. Jean J. Schuenenman of U.S. Public Health Service spoke on the activities of his organization in air pollution and the problems encountered throughout the United States. The Section also held a noise symposium in May. This symposium was jointly sponsored with several other organizations in the Houston area and was well received and attended.

Tennessee Valley Section

This Section held a recent meeting to coincide with the meeting of the AEC Fellowship Awards Committee. Dr. Anna Baetjer, Dr. Allen Brandt, W. G. Hazard, T. F. Hatch, Nathan Van Hendricks, Harry Schulte, Dr. Leslie Silverman, and Dr. Donald M. Ross, members of the Committee, were guests of the Section at the meeting and dinner. Mr. Hatch spoke extemporaneously on the necessity for combined teamwork of various professional disciplines in order to solve the occupational health problems of present-day industry.

Georgia Section

New officers of this Section are Dr. A. A. Weinstein, President; Dr. Harry E. Dickson, President-Elect; and Gwen Dekle, Secretary-Treasurer. At the meeting in which these officers were installed, Dr. Roy O. McCaldin spoke briefly on the current air pollution research being conducted at the Robert A. Taft Sanitary Engineering Center. The talk was preceded by a film "Report on Smog."

North Texas Section

New officers of this Section are Walt Grizzle, President; G. M. Kintz, President-Elect; and Charles W. Reed, Secretary-Treasurer. Recent meetings of this Section included a talk by Mr. Eugene Spivak on activities of the Food and Drug Administration. At a later meeting a very interesting film on heart surgery was featured.

A major activity of this Section was participation in the Health Fair in Dallas several months ago. The local Section, in cooperation with the U.S. Bureau of Mines, put on a demonstration called "The Air We Breathe." In this demonstration a white rat was asphyxiated with hydrogen sulfide, then revived by artificial respiration. The booth received wide local publicity as well as movie news coverage.

Michigan Section (Detroit)

Recent speakers at meetings of this Section were Elmer P. Wheeler who spoke on "Current Trends in the Profession of Industrial Hygiene and the A.I.H.A."; Dr. H. E. Stokinger reporting on "Toxicological Interaction of Air Contaminants"; and Dr. Arthur Vorwald discussing siderosis and the industrial medical and hygiene conditions observed during his recent trip to South Africa.

Western New York Section

New officers of this Section are Richard F. Scherberger, President; Dr. Louis J. Casarett, President-Elect; and Abe S. Levy, Secretary-Treasurer. At the annual meeting the Section considered establishing an award in memory of Herb Miller and dedicated to promoting the interest of students in industrial hygiene. The Section is also planning an active program for the stimulation of membership in AIHA and the promotion of recognition of industrial hygiene by industry, labor, and the general public. They have conducted a survey on the preference of the members for meeting place, time, and subject matter. Without details, we can comment that the interest and views of the members are quite widespread. This Section is also to be congratulated for their fine support of the 1960 Industrial Health Conference.

Personnel Notes

Dr. Thomas F. Mancuso, Chief, Division of Industrial Hygiene, Ohio Department of Health, received the Award of Merit at the annual banquet of the American Conference of Governmental Industrial Hygienists which was held in Rochester, New York. Dr. Mancuso was cited for his "substantial contribution to the methodology of investigation of chronic occupational disease and for his outstanding administration of governmental industrial hygiene agencies." He was especially commended for his use of social security records as an epidemiologic tool in determining the long-term effects of occupational exposures.

Dr. Bryce B. Reeve retired March 28, 1960, from his position as medical director of Standard

Oil Company (Indiana). He had been with the company for more than 36 years.

Dr. Gilbeart H. Collings, Jr. was appointed to succeed Dr. Reeve as medical director of Standard Oil Company (Indiana).

Paul D. Halley has been promoted to the position of director of industrial hygiene, Standard Oil Company (Indiana).

Dr. Marvin R. Alberg has been promoted to the position of chief physician in charge of the general office medical department, Standard Oil Company (Indiana).

American Standard Radiation Symbol

EARLY IN 1957, the new Nuclear Standards Board of the American Standards Association authorized a project designated as N-2 for the development of general and administrative standards in the field of nuclear energy. The Atomic Industrial Forum, Inc. agreed to be the sponsor for this project. The AIHA was represented on the N-2 commitee by J. A. Martin along with 15 other professional groups with interest in this field. The committee first met in July, 1957, and the American Standard Radiation Symbol N2.1-1960 was approved by the American Standards Assoication on March 1, 1960.

Over a period of years, a radiation symbol, consisting of a trefoil (three-bladed propeller) in reddish purple (magenta) color on a yellow background, has become accepted by nearly all organizations engaged in work with radiation or radioactive materials. At the present time there are many variations in the design of the symbol and in its dimensions. There are also some differences in the range of colors used. The radiation symbol described in the American Standard N2.1-1960 had its beginning at the University of California Radiation Laboratory in the latter part of 1946. Various designs were considered in an attempt to show degree of hazard, type of activity, or other information; but for simplicity, the design finally chosen was an upside down version of the symbol given in the American Standard. A light blue was initially selected as the background color for the symbol, because it was a color not often seen in areas where the symbol was likely to be used. Sometime later the background color was changed to yellow because it had been determined scientifically to be one of the most attention-getting of the colors. In September 1953, the American Standards Association published a new safety color code for Marking Physical Hazards and the Identification of Certain Equipment. This code listed purple as the proper color to designate radiation hazards and stated that it should be used in combination with yellow. The radiation symbol described in American Standard N2.1-1960 has been recommended by the National Committee on Radiation Protection and Measurement. It was submitted at the International Standards Organization in Geneva, May, 1960. To date, it is known to have been approved officially by 13 agencies of government, and its use is mandatory for denoting the hazards due to radiation sources which are under the control of these agencies. The proposed symbol has also been adopted in one form or another by numerous industrial, educational, research, medical and other organizations which are engaged in radiation work. In order to confirm that there exists a national concensus in favor of the proposed standard, the Committee circulated copies of the proposal to more than 450 industrial, educational, governmental and other organizations which are engaged in atomic energy and/or radiation work. The results of this survey indicated an overwhelming acceptance of the standard.

The "American Standard Radiation Symbol," N2.1-1960 contents are: (1) Scope, (2) Standard Radiation Symbol, (3) Color Code for Symbol, (4) Restrictions on the Use of Symbol. It is available for 50¢ per copy from the Atomic Industrial Forum, Inc., 3 East 54th St., N. Y. 22, N. Y.

Papers for 1961 Annual Meeting

THE Twenty-second Annual Meeting of the American Industrial Hygiene Association will be held in Detroit, Michigan in April, 1961. This is a very important meeting in the history of our Association, and a great amount of planning and effort have already gone into it in order that it may be a true landmark in and worthy of our profession. To this end, the planning of the technical programs has begun, and all persons having or anticipating papers for presentation at this meeting are invited to submit preliminary notice of such papers to the Session Arrangers.

Laboratory or field work, and writing on many potential papers will have only begun at this date, however preliminary notice of your intention to submit a paper will assure you of full consideration for a place on the 1961 program. Include a tentative title and a brief summary statement of the work to be presented. Where necessary the summary may be a projection of plans but such results as are available should be mentioned.

Early submission of completed manuscripts will hasten the processes of review and early publication. Under the policies of AIHA all papers presented at the annual meetings are automatically submitted to the AIHA Journal with first rights of publication reserved to this Journal. A few exceptions to this policy may be granted by the President, President-Elect, or the Editor for unusual and compelling reasons, however requests for such action should be received prior to acceptance of the paper. In most instances, the Journal will wish to review the paper before granting a release.

Submit your preliminary notice or completed manuscript as soon as possible to the appropriate Session Arranger:

Air Pollution

Mr. Robert T. Pring
American Wheelabrator & Equipment Company
Mishawaka, Indiana

Engineering

Mr. Maurice F. Clay
General Electric Company
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Toxicology

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Noise

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